INVESTIGATIONS ON INDIGENOUS SEED FATS AND THEIR COMPONENT FATTY ACIDS

THESIS

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R E S U M E

The work described in the thesis consists of two parts:

(A) Part I deals with the analysis of some indigenous seed fats.

(B) Part II describes the study of some reactions of 10-undecenoic acid.

(A) Nineteen oils from indigenous seeds belonging to different botanical families have been analysed for their fatty acid composition by using the chromatographic and spectroscopic techniques. The important features of each fatty acid analysis are enumerated below:

(i) Out of the four seed oils of the family Apocynaceae Wrightia tomentosa seed oil contained an unusual unsaturated hydroxy acid as a major component. The structure of this acid was established as 9-hydroxy-cis-12-octadecenoic (strophanthus) acid. Wrightia oil is an unusually rich source of strophanthus acid (67%).

(ii) The two seed oils of the family Malvaceae and Sterculiaceae were found to contain malvalic acid (11.7%) and sterculic acid

(3.4%) respectively. These acids were identified by i.r., n.m.r., and combined TLC-GLC techniques.

(iii) The five seed oils of the family Coniferae were found to be usual oils belonging to oleic-linoleic type of natural oils.

(iv) The seed oils from families (Solanaceae, Compositae, *Oleaceae*, *Chenopodiaceae*, *Verbenaceae*, *Capparidaceae and* Euphorbiaceae) were found to contain usual fatty acids. Nyctanthus seed oil (Oleaceae) contains oleic acid in a high proportion (68.9%). Hyoscyamus and Jatropha seed oils contain 71.1 and 69.5% of linoleic respectively.

(B) The work discussed in Part II of the thesis describes *the results of the following reactions:*

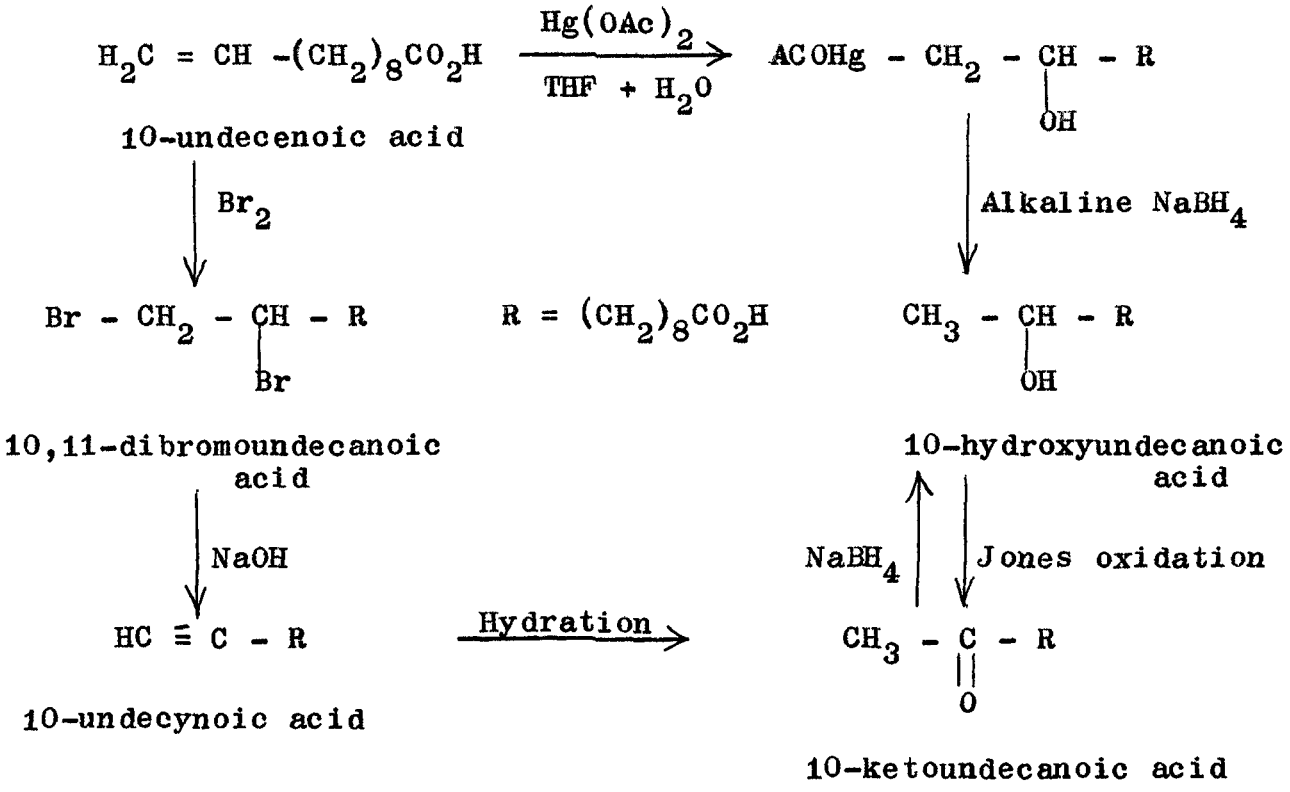
(a) The Solvomercuration-demercuration studies has been carried out with 10-undecenoic acid using mercuric acetate in presence of a 50:50 tetrahydrofuran-water medium, followed by in situ reduction of the oxymercurial to obtain the hydration product characterized as 10-hydroxyundecanoic acid. The above reaction when carried out in presence of methyl, ethyl and isopropyl alcohols and ethylene and propylene glycols as nucleophilic solvents resulted in the formation of the corresponding alkoxy and hydroxy ethers respectively. Use of acetonitrile yielded

the amination product. It is found that in all cases except isopropyl alcohol reaction proceeded smoothly in the Markovnikov direction.

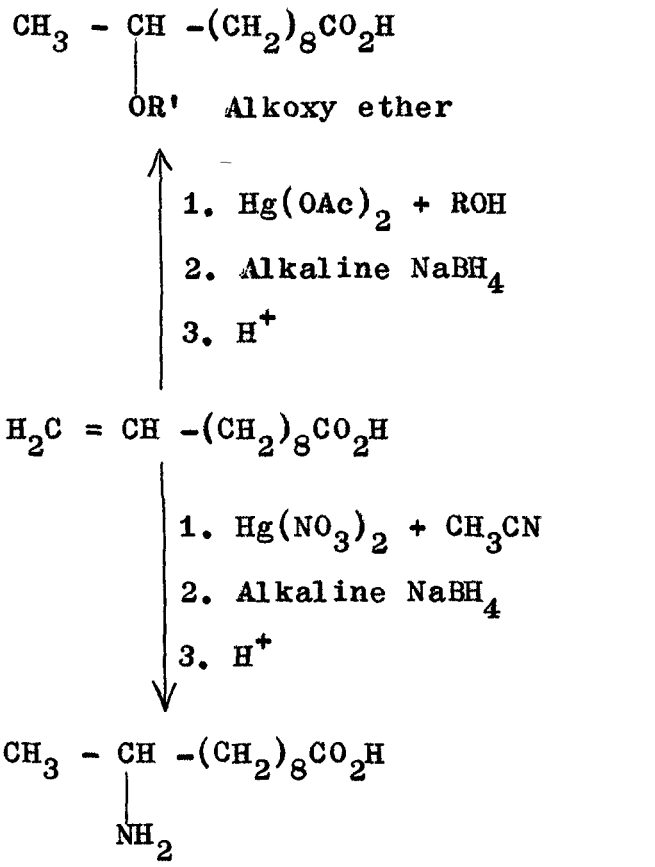
An attempt to oxidize the hydroxy ethers (XIX) and (XX) with Jones reagent led to a very interesting result. Instead of the corresponding oxidation product, 10-ketoundecanoic acid is obtained which represents an unusual feature of the reaction.

The results of Solvomercuration-demercuration reactions are shown in chart - 1, 2 and 3.

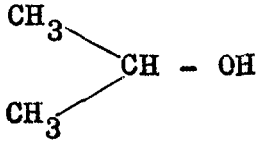
C H A R T - 1.



C H A R T - 2.

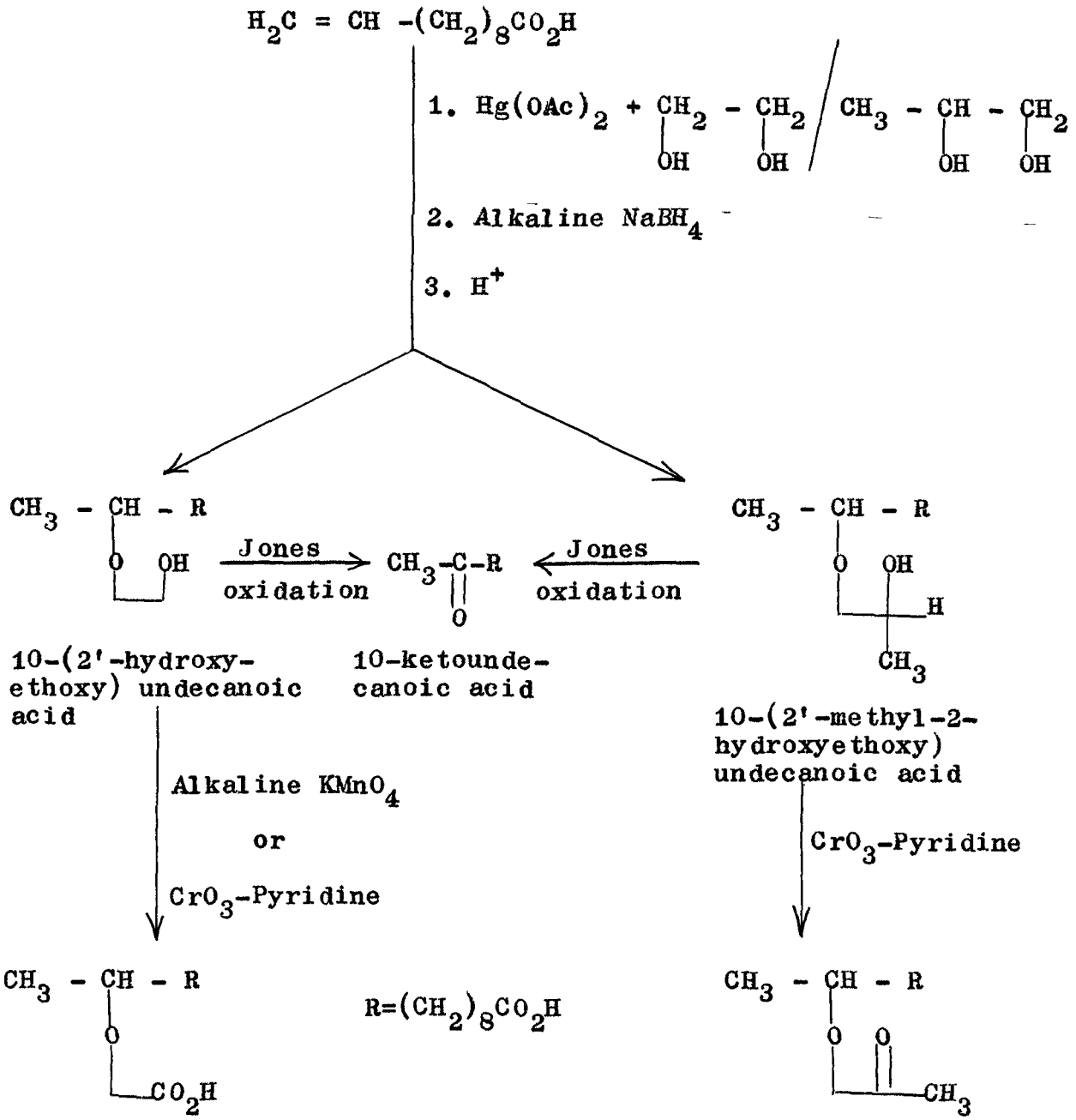


10-aminoundecanoic acid

R = CH_3OH, CH_3CH_2OH, 

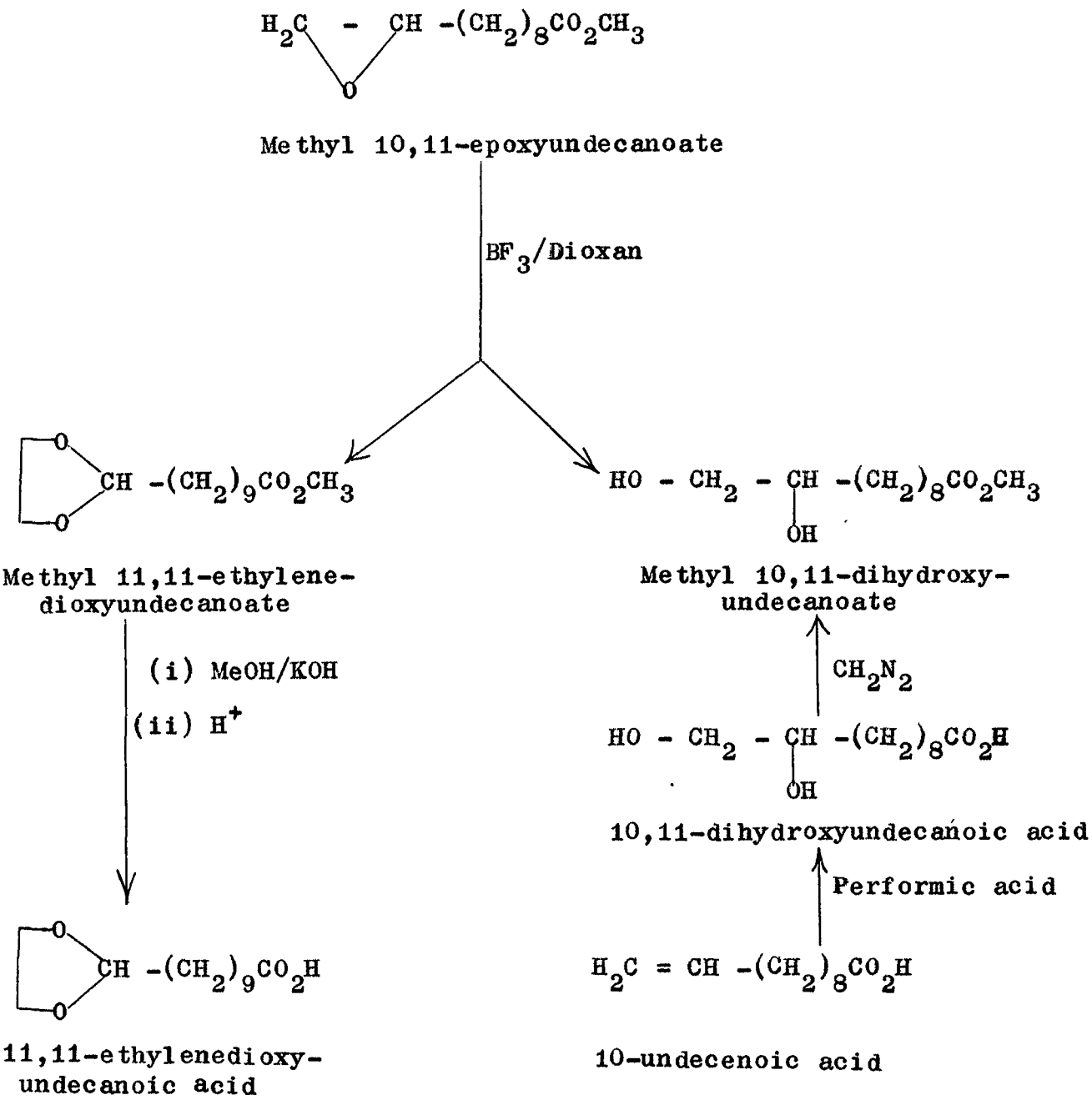
R' = $-CH_3$, $-CH_2-CH_3$

C H A R T - 3.


H2C = CH -(CH2)8CO2H
1. Hg(OAc)2 + CH2 - CH2 / CH3 - CH - CH2
OH OH OH OH
2. Alkaline NaBH4
3. H+
CH3 - CH - R
O OH
Jones oxidation
CH3-C-R
O
Jones oxidation
CH3 - CH - R
O OH
H
CH3
10-(2'-hydroxy-ethoxy) undecanoic acid
10-ketounde-canoic acid
10-(2'-methyl-2-hydroxyethoxy) undecanoic acid
Alkaline KMnO4
or
CrO3-Pyridine
CrO3-Pyridine
CH3 - CH - R
O
CO2H
R=(CH2)8CO2H
CH3 - CH - R
O O
CH3

(b) The borontrifluoride catalysed rearrangement of methyl 10,11-epoxyundecanoate in dioxan resulted in the formation of an unexpected cyclic product, methyl 11,11-ethylenedioxyundecanoate and the expected methyl 10,11-dihydroxyundecanoate. The reactions are represented in Chart - 4.

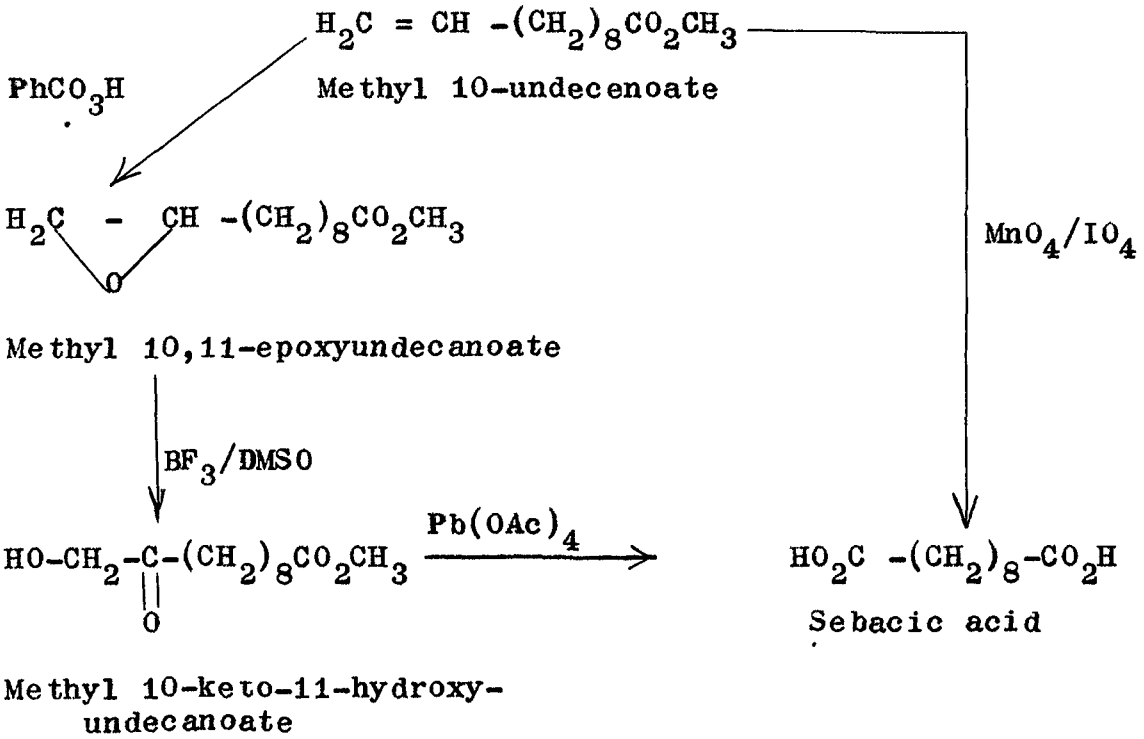
C H A R T - 4.



It is interesting to note that the attempted rearrangement reaction in case of a terminal epoxy fatty ester leads to an abnormal cyclic product rather than the usual carbonyl derivative. A tentative mechanism for this unusual reaction has been suggested in the text.

The BF_3 catalysed dimethyl sulfoxide oxidation of methyl 10,11-epoxyundecanoate resulted in the formation of exclusively one isomeric α-ketol, methyl 10-keto-11-hydroxyundecanoate. The identity of the product and the positions of hydroxyl and keto functions were established by spectral and degradation studies of the product. The reactions are summarised in Chart - 5.

C H A R T - 5.

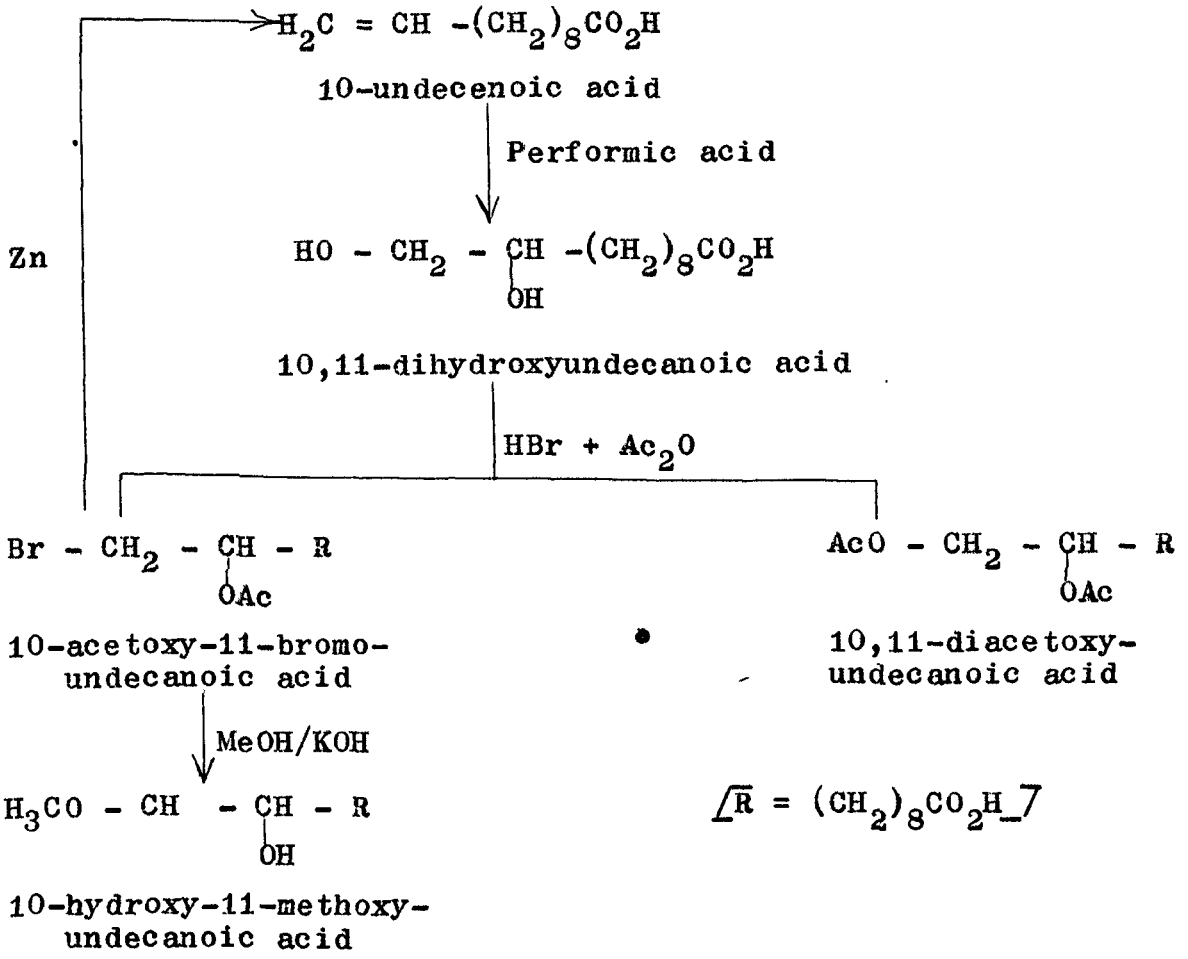


(c) The reaction of hydrogen bromide in acetic anhydride with 10,11-dihydroxyundecanoic acid resulted in the formation of 10-acetoxy-11-bromo and 10,11-diacetoxyundecanoic acids characterized as minor and major products respectively.

The interesting features of the above reaction are (i) Non-formation of the keto acid, reported earlier in the case of nonterminal dihydroxy fatty acids and (ii) methanolic potash hydrolysis of bromoacetoxy acid afforded 10-hydroxy-11-methoxyundecanoic acid instead of the expected bromohydroxy acid.

The above reactions are given in Chart - 6.

C H A R T - 6.



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This is to certify that the work described in this thesis is the original work of the candidate done under my supervision. The thesis is suitable for submission for the award of Ph.D. degree in Chemistry.

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C O N T E N T S

INTRODUCTION

In the plant kingdom about 2,50,000 known species of higher plants are known to exist. These comprise about 330 known plant families. Much of the work on the fatty acid composition of seed fats reported before 1960 are no longer considered highly reliable. Quite divergent results of the reinvestigation of the earlier work have appeared in recent years due to the use of new methods of oil analysis. It is now well established that oils containing unusual functional groups often give unexpected responses to analytical procedures in frequent use. This has led to significant advances in the separation, isolation and identification procedures employed in the field of fatty acid chemistry.

The variety of products of oils and fats used in trade and industry has given rise to a systematic survey of the physical, biological and chemical properties of seed oils. Extensive programmes have been initiated in various countries to search new industrial raw materials among the many oil-bearing plants that have little or no study of their chemical composition. The present trends indicate increasing use of seed oils as sources of chemical intermediates that can be

purified and processed into products of controlled quality. Desired oils for processing might contain a high concentration of an unknown acid having structure suitable for the preparation of useful derivatives. Examples of such structures include oxygenated acids (hydroxylated, ketonic or epoxydized), unusual unsaturation (conjugated, acetylenic or positional isomers), branched or cyclic acids, and combinations of these structures. The development of spectroscopic equipments and modern separation techniques have contributed much to our recognition of the variety of fatty acids in existence and of their molecular structure.

During the last decade new and interesting reactions of fatty acids have been described that provide new route to the synthesis of a variety of fatty acid derivatives. Interest in this field of fatty acid chemistry has been created by the attempts to study the reactivity of the polyfunctional fatty acids and to find clues for the biosynthesis of essential fatty acids. The chromatographic procedures of separation and spectral methods of structure determination have encouraged research in these aspects of fatty acid chemistry.

Fundamental and applied research on indigenous oils and fats is now considered to be essential for raising the economy and for maintenance of nutritional standards of the people of

developing countries. A search for the major constituents of seed oils offers promise of successful commercial exploitation of their major component acids. Keeping in view these objectives it is considered of interest to make a systematic study of the indigenous seed fats and to study the reactions of fatty acids possessing novel structures. A successful achievement of this aim of research would help to exploit the minor seed oils available in the country as well as to study the chemistry of unusual fatty acids.

P A R T - I

Component Fatty Acids of Seed Fats

Our present knowledge about the fatty acid composition of seed fats is comparatively of recent origin. In the year 1965 it was reported[1] that from all higher plants lipids of only about 900 species have been studied for their average composition of total fatty acids. This is still only a tiny cross-section of the total number of known species available in nature. As a consequence of the plant material readily available for study, a large segment of seed lipids could not be chemically characterized. Much of the work reported earlier in the literature was based on classical methods that are no longer considered highly reliable. It has rightly been pointed[2] out that the lipid composition of no plant or plant seed is completely known. During the last decade the significant advances in the methods of isolation, separation and structure determination have led to the discovery of a variety of unusual fatty acids in seed fats. In the course of chemical compositional studies on seed oils from more than 2600 species of plants, a number of unusual acids were isolated and characterized by Wolff and coworkers in the Northern Regional Research Laboratories, Peoria, U.S.A. A review on seed lipids written by

Wolff[3] contains a detailed account of the occurrence of fatty acids in natural fats. A similar type of screening programme of indigenous oil-bearing seeds has also been initiated in U.K. and Canada by F.D. Gunstone and H.Y. Hopkins, respectively. The newly discovered acids in recent years are found to occur as major components of seed oils and have structural features that mark them as quite unusual according to earlier concept. The number of known fatty acids of lipid origin is now over 300 and the number is increasing rapidly. It is surprising that till 1965 only 80 natural C18 fatty acids were known out of which more than half were discovered during the period 1960-65. Keeping in view the line of work described in this thesis a brief account is given here of the occurrence, detection and methods of analysis of fatty acids in seed oils.

(a) Unusual Olefinic acids.

The most common unsaturated acids are oleic (cis-octadec-9-enoic), linoleic (all cis-octadec-9,12-dienoic) and linolenic (all cis-octadeca-9,12,15-trienoic). Besides these acids a number of olefinic acids ranging in chain length from C14-C24 and containing 1-4 double bonds have been reported in recent years. Some general comments about the occurrence of olefinic acids are now given to illustrate the complexity observed in the natural fatty acids.

Monounsaturation among C18 acids has been found at positions, 3,5,6 and 11. Monounsaturation at the 7th position of the fatty acid chain has not so far been demonstrated in a seed oil. Until recently trans monoenes had not been reported to occur naturally in vegetable oils. Cis configuration of bonds is generally accepted as the pattern characteristic of unsaturated seed oils. Polyolefinic unsaturation in all known seed oil acids classically follows one of the two patterns. Either the unsaturation is conjugated (-CH=CH-CH=CH-), or any double bond present is separated by a single methylene group in the hydrocarbon chains (-CH=CH-CH_2-CH=CH-). The first well authenticated example of structure in seed oils that deviate from this pattern in having more widely separated double bonds, (-CH=CH-$(CH_2)_{n>1}$ -CH=CH-), has been found in the past few years. The isolated double bonds nearest to the carboxyl end of the acid so far encountered are either in the 3 or 6-position and may be cis or trans in geometrical configuration; other centres of unsaturation in the molecule are in the conventional position. Both positional and geometrical isomers are known for the usual all cis-polyenoic acids with one methylene group separating neighbouring double bonds.

Fatty acids with conjugated unsaturation recently discovered in many seed oils illustrate the complexity and variety of naturally occurring polyunsaturated fatty acids (PUFA). Out

of the 330 plant families, conjugated fatty acids have been identified in the seed oils of 11 families (Dicotyledons) of plants. No conjugated acids have been found yet in the monocotyledons. Surprisingly the simple conjugated diene acids are apparently much less common in seeds than are the hydroxy dienes, the trienes or the acetylenic acids. About more than twenty fatty acids with conjugated unsaturation have been discovered in the seed oils within the last few years. It is likely that more will be found because of the increasing interest in fat components and the availability of new techniques.

Until 1964 only one acetylenic seed oil fatty acid, tariric (octadec-6-ynoic) was known. Since then a number of acetylenic fatty acids have been discovered in the oils of plant families Olacaceae, Compositae, Santalaceae and Simaruboceae. While discussing the distribution pattern of acetylenic acids in a number of Santalaceae species, Hopkins and Chisholm[4] have suggested that the presence or absence of a particular acid may constitute a useful chemotaxonomic character of plant classification. Allene functions are uncommon in nature and only recently have been discovered in two seed oils belonging to Labiatae[5] and Euphorbiaceae[6]. The more interesting feature of the recently discovered unusual olefinic acids is that a number of polyfunctional unsaturated acids such as containing dienol,

enynol, epoxy, cyclopropene groupings have also been found to be present in seed oils.

(b) Oxygenated fatty acids.

New oxygenated fatty acids containing epoxy, hydroxy, and keto functions have been found to occur naturally in seed oils. Only in 1954 was the first natural epoxy acid, vernolic acid isolated and structurally characterized as cis-12,13-epoxy-cis-9-octadecenoic acid. Two additional new epoxy acids have also been discovered. These epoxy acids may be regarded as derivatives of oleic, linoleic and linolenic acids respectively, in which one of the usually present double bond is epoxidized.

In a current speculative article on the role of epoxy acids as intermediates in the biosynthesis of polyunsaturated fatty acids, Gunstone[7] mentioned that epoxy acids occur in more than 40 species from twelve different plant families. Recently Krewson[8] has forecasted the possibility of the discovery of epoxy acids corresponding to epoxidation at 9 and/or 12-olefinic bond of linolenic acid. Further he suggested that the existence in some seed oils of other than 18 carbon epoxy fatty acids and of epoxides containing more than one epoxy function cannot be ruled out. Sometimes two or more of the four known epoxy acids occur in the same seed oil.

The only hydroxylated vegetable oil available at present in rather large commercial quantities is castor oil belonging to Euphorbiaceae which contains ricinoleic acid (12-hydroxy-cis-9-octadecenoic) as a major component acid. Recently Siddiqui et al.[9] have identified this acid as a major component of Hiptage seed oil. An isomer of ricinoleic acid, strophanthus acid (9-hydroxy-cis-12-octadecenoic) was first reported by Gunstone[10] in seed oils of the genus Strophanthus, family Apocynaceae[11a]. Very recently in our laboratory this acid has been identified in the mixed fatty acids of Wrightia tinctoria and W. tomentosa,[11b] family Apocynaceae. New sources of 9-hydroxy-cis-12-octadecenoic acid are three seed oils of Apocynaceae recently reported by Powell and coworkers[12]. A higher homologue of ricinoleic acid, lesquerolic acid (14-hydroxy-cis-11-eicosenoic) has been discovered in the genus Lesquerella of the family Cruciferae by Smith and coworkers[13].

In 1960 the first representative, dimorphecolic acid (9-hydroxy-trans-10, trans-12-octadecadienoic) of a new class of naturally occurring seed oil acids, was reported by Smith and coworkers[14], in which hydroxy group is alpha to a conjugated diene grouping. Other conjugated hydroxy dienoic acids were soon found in other seed oils mainly in the plant family Compositae. Morris et al.[15], Chisholm and Hopkins[16] found indepen-

dently the mixture of 9-hydroxy-10,12-, and 13-hydroxy-9, 11-octadecadienoic acids in several seed oils. These acids were found to be either cis, trans or trans, cis in configuration. Later the 13-hydroxy-9,11-acid (coriolic) was reported by Tallent et al.[17] to occur itself without the 9-hydroxy-10,12-isomer in a species of Coriariaceae. This pair of hydroxy acids were characterized as 9-hydroxy-trans-10, cis-12-and 13-hydroxy-cis-9, trans-11-octadecadienoic acids. Thus the hydroxy diene acids known so far (except cis, cis acid) consist of three isomers with the configuration cis-9, trans-11; trans-10, cis-12; and trans-10, trans-12 respectively.

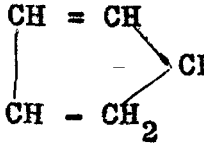
A non-conjugated hydroxy diene acid, densipolic (12-hydroxy-cis-9-cis-15-octadecadienoic acid) has been discovered by Smith and coworkers[18] as a major constituent in the Lesqurella densipola seed oil glycerides. Very recently Smith and Wolff[19] have discovered a very unusual hydroxy triene acid, α-hydroxy linolenic acid, (2-hydroxy-cis-9, cis-12, cis-15-octadecatrienoic) in the seed oil of Thymus vulgaris, family Labiatae.

Hydroxy acetylenic acids tend to occur in fairly large proportion in some of the species that have acetylenic acids. Ligthelm[20] isolated 8-hydroxy-ximenynic acid from ximenia oil. Riley[21] later discovered 8-hydroxy-isanic adid from Ongokea seed oil. An acetylenic analogue of dimorphacolic acid, helenynolic acid (9-hydroxy-trans-10-ene-octadec-12-ynoic) was discovered

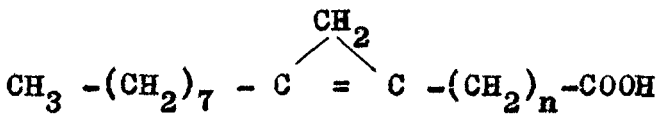
by Powell and coworkers[22] in Helichrysum seed oil of Compositae. Other hydroxy acetylenic fatty acids have also been identified in Ongoken seed oil by Gunstone and Sealy[23] and also by Morris[24]. Powell et al.[25] detected and identified several new hydroxy acetylenic acids in Acanthosyris oil, Santalaceae. One of these is the first C17 fatty acid (7-hydroxy-trans-10,16-octadecadiene-8-ynoic) to be found in quantity in a seed oil[26]. It appears that hydroxy acetylenic acids are nearly as numerous as hydroxy olefinic acids in seed oils but may not occur in as many plant families.

Fatty acids containing ketone functions are rarities in natural lipids of plant origin. The only ones whose complete structures have been recorded in the literature previously are 4-oxo-cis-9, trans-11, trans-13-octadecatrienoic acid (∝-Licanic), and 9-oxo-trans-10, trans-12-octadecadienoic acid. More recently Smith[27] has reported the presence of three keto fatty acids with unusually long carbon chain in the seed oil of Cuspidaria pterocarpa, Bignoniaceae. These keto acids combine two features rarely found in lipids of higher plant, ketone function together with chain lengths longer than C22. These keto acids are 15-oxo-cis-18-tetracosenoic, 17-oxo-cis-20-hexacosenoic and 19-oxo-cis-22-octacosenoic acids.

(c) Cyclic fatty acids.

Among the naturally occurring cyclic fatty acids, the cyclopropene and cyclopent-2-ene acids have been reported in seed oils. Chaulmoogra oil and some related seed oils of the family Flacourtiaceae contained acids having a cyclopentene group,  The C16 (Hydnocarpic) and C18 (Chaulmoogric) acids are more common, but lower member and more unsaturated C18 acid, gorlic (Δ^6-derivative) are also known. All these acids are obtained in dextrorotatory form.

The Sterculic acid (Ia) was first characterized by Nunn[28] in (1952). This acid is now known to occur in seed oils or fruit fats of the Malvaceae, Sterculiaceae, Tiliaceae, and Bombacaceae families and to be accompanied sometimes by malvalic acid (Ib) and their dihydro derivatives.



(Ia) sterculic acid (n=7); (Ib) Malvalic acid (n=6)

Very recently Jevans and Hopkins[29] have reported an acetylenic cyclopropene acid 'Sterculynic' (8,9-methyleneoctadec-8-ene-17-ynoic) in the seed oil of Sterculia alata. Morris et al.[30] have discovered 2-hydroxy Sterculic acid from the seed oil of Pachira insignis and Bombacopsis glabra. Morris and coworkers[31] have also

reported a cyclofuranoic acid, 8(5-hexylfuryl-2-)-octanoic in the seed oil of Exocarpus Cupressiformis, family Santalaceae.

From the foregoing account of the natural occurrence of novel fatty acids in seed oils, it appears that most of the unusual fatty acids also co-exist in the same seed oil. This suggests strongly that the various acids containing unusual functions are related to one another biosynthetically.

Isolation and characterization of fatty acids.

Screening analysis of oils from seeds of a number of plant families carried out in recent years have shown the presence of a variety of unusual fatty acids that interfere with the application of standard methods in their isolation and characterization. These acids possess unusual functions such as hydroxy, epoxy, keto, allenic, acetylenic, cyclopropenoid and conjugated unsaturation. Surprisingly a particular seed oil analysed by different workers has been found to contain more and more unusual minor component acids. This complexity inherent in the natural fatty acids has created many problems in their detection, separation and structure determination. Prior to 1955, the separation methods were based on the distillation and crystallization techniques such as ester fractionation, urea-adduct formation, low temperature crystallization and lithium/lead-salt separation of fatty acids. These con-

ventional procedures have now been superceded by chromatographic and counter-current distribution techniques. The development of chromatographic techniques have completely revolutionalized assays of the fatty acids. First developed by the use of absorption column chromatography, reversed-phase and argentation (column/thin-layer) and liquid-vapor phase technique were then rapidly adopted for the analysis of fatty acids. Amongst the various techniques TLC and GLC have gained more prominence in the field of fatty acid analysis. The first application of argentation TLC was made simultaneously by Morris[32] and Barrett and coworkers[33]. Development of argentation column chromatography by DeVries[34] and reversed-phase partition chromatography by Howard and Martin[35] are the two other chromatographic procedures widely used in the separation of fatty acids. The former method is applied for the separation of cis and trans isomers of fatty acid esters and of esters according to the degree of unsaturation. Reversed-phase technique is mainly used for the separation of fatty acids according to chain length. The use of glycol complexing agents such as boric acid, sodium borate and sodium arsenite for the separation of erythro and threo isomers of polyhydroxy fatty acids has been reported by Morris[36]. Very recently Bioque[37] developed an indirect method for the identification of cis and trans-epoxy compounds using a resin as a specific hydration agent for epoxy group. The corresponding diol is then characterized by boric acid-TLC.

During recent years a few diagnostic TLC spot tests have been developed for the detection of unusual function. Freeman and coworkers[38] have used a spot test for the detection of hydroxyl group using cellulose thin-layer plate, the reagent 4-(p-nitrobenzyl) pyridine and spraying solution sodium carbonate. A development of deep blue or purple spot on chromatoplate detects the presence of hydroxyl group. Another spot test of particular interest to fat chemist is the picric acid TLC for the detection of epoxy function. Fioriti and Sims[39] have used picric acid as a spraying reagent which develops orange spot on the chromatoplate. Their developing system was petroleum ether-diethyl ether-acetic acid (75:25:1; V/V). Very recently Davis and coworkers[40] have successfully used the spraying reagent 2,4-dinitrophenyl hydrazine hydrochloride for detecting the carbonyl group in oxygenated fatty acids. In this procedure of TLC they have used silica gel sheets (Eastman chromatogram silica gel sheets) and a mixture of hexane-ether (9:1; V/V) as the developing solvent. Yellow hydrazone spots develop without heating.

Although TLC is capable of fractionating fatty acid mixtures according to unsaturation, chain length, types of functional groups and geometry of double bonds, direct quantitation is seldom achieved. Therefore, the application of GLC in recent years has come to prominence. A combined or integrated TLC and GLC technique can only permit a complete resolution and quantification of all compo-

nents of a mixture of fatty acids under investigation. GLC is capable of the greatest sensitivity, the highest resolution and accuracy in the analysis of fatty acid composition of natural oils and fats. GLC provides the most effective method of quantitative analysis of fatty acid mixtures and can give considerable information about acids of unknown structure.

Although GLC is a very useful tool for fatty acid analysis it suffers from some limitations. Conjugated trienoic acids undergo stereomutation and migration of the triene unit. Cyclopropene acids and hydroxy conjugated dienoic acid tend to decompose unless the temperature is carefully controlled. Also some fatty acids like methyl linoleate and methyl malvalate are either unresolved or incompletely resolved on both polar and non-polar columns. In recent years the use of preparative GLC has made it possible the isolation of pure fractions from a complex mixture. The collected fractions can be chemically modified and then re-examined by chromatographic analysis. Keto compounds can be converted to N,N-dimethyl hydrazides, or reduced to hydroxy compounds. Hydroxy esters can be oxidized to keto esters, acetylated or converted to their trimethyl-silyl ethers or trifluoroacetyl isopropylidine derivatives.

The modern methods of seed oil analysis include the above mentioned chromatographic techniques as well as the spectroscopic

methods. Out of the four major forms of spectroscopy only u.v. and i.r. have been widely used in the routine analysis of seed oils. A direct u.v. spectrum of an oil showing maxima in the region 200-400 mu gives positive indication for the presence of conjugation in the component fatty acids. U.v. analysis of oils or their acids after alkali isomerization are the standard procedures, though not frequently used,for the detection and estimation of conjugated fatty acids. The principal use of i.r. spectroscopy has been to detect and to measure trans unsaturation (10.3 μ). It is of particular value in the detection of unusual functional groups in the fatty acids. The use of n.m.r. and mass in the structure determination of fatty acids is described in part II of the thesis.

Despite the development of physical methods of fatty acid identification, chemical methods are also necessary to confirm the structure of fatty acid. In a structure of unknown acid the problem arises in the exact determination of the position of unusual functions, type of unsaturation and its geometry. Among the natural fatty acids discovered in recent years, oxygenated and cyclopropene acids have attracted considerable attention. The unsaturated hydroxy acids may possess either of the two conjugated dienol groupings like $-CH=CH-CH=CH-\underset{OH}{CH}-$; $-\underset{OH}{CH}-CH=CH-CH=CH-$. Similarly in the conjugated enynolic isomeric acids containing $-CH=CH-C\equiv C-\underset{OH}{CH}-$ and $-C\equiv C-CH=CH-\underset{OH}{CH}-$, the difficulty arises to determine whether the hydroxyl group is

alpha to the double bond or triple bond. Further the co-occurence of any two HBr reacting fatty acids (containing α - hydroxy conjugated diene and cyclopropenoid moieties) poses a problem for the analyst.

The foregoing structural problems encountered during the course of fatty acid identification have led to the development of elegant chemical methods that include the following standard organic reactions.

(i) Hydrogenation
(ii) Hydroxylation
(iii) Oxidative degradation
(iv) Partial hydrogenation and partial oxidation
(v) Diel's Alder reaction
(vi) Hydrogen bromide reaction.

Besides the above reactions the following ones have been found to be more useful for solving special type of structural problems:

(1) HBr titration (method of Harris and coworkers[41]) of acids before and after reduction by $LiAlH_4$ to distinguish between the epoxy and cyclopropenoid acids;

(2) Cleavage of saturated hydroxy acid by solid potassium permanganate for the location of hydroxyl group;

(3) Dehydration of a dienol to all trans triene acids by

treatment with glacial acetic acid for establishing the presence of allylic hydroxyl grouping;

(4) Reduction of secondary alcoholic group -CHOH- to $-CH_2-$ by hydriodic acid and phosphorus for determining the chain length;

(5) Reductive removal of hydroxyl group by the reduction of acid tosylate with $LiAlH_4$ followed by oxidative degradation of unsaturated acid by permanganate periodate for locating the position of unsaturation;

(6) Lipoxidase catalysed isomerization to conjugated acids for detecting cis, cis-methylene interrupted double bonds.

PRESENT WORK

Analysis of Indigenous Seed Fats.

Although India is one of the major producer of oil seeds, the production of this commodity is not keeping pace with the rising domestic needs for edible and non-edible oils. There are vast resources of herbaceous and higher plant minor oil seeds available wildly, which have not been exploited so far. During recent years the analysis of seed fats from an extensive sampling of the plant kingdom has revealed that a large number of uncultivated growing species yield seeds whose oils are of unusual composition as compared to the common seed oils. Quite a large number of less familiar fatty acids possessing novel structures have been discovered in recent years by the application of the modern chromatographic and spectroscopic methods of oil analysis. A programme of a systematic screening of oil-bearing seeds had been initiated in these laboratories for a long time. A number of seed oils belonging to different plant families have already been analysed for their component fatty acids. Keeping in view the easy availability of minor oil seeds in the vicinity of the University campus, it was considered of interest to collect oil-bearing seeds for chemical analysis. The work described in this thesis deals with the fatty acid analysis of a few seed fats from the indigenous sources.

Materials and Methods.

(i) Source of Oil Seeds. Seed samples for the present study were obtained from numerous sources, including the seed firms and collections from plants grown in and around the University campus.

(ii) Oil extraction. Oil was extracted from crushed seeds with petroleum ether in a soxhlet extractor until no more oil was removed.

(iii) Preparation of mixed fatty acids. Seed oil was refluxed with ethanolic potassium hydroxide. The unsaponifiable material was removed and the free fatty acids were obtained in the usual manner. Wherever necessary saponification was carried out under nitrogen and samples were stored at low temperature in a nitrogen atmosphere.

(iv) Methyl esters. Esterification and trans-esterification were carried out as follows, except where specified. Fatty acid samples were refluxed for 1 hr in a large excess of methanol containing 1% sulphuric acid. In each case, resulting mixtures were diluted with water, chilled in an ice-bath and then extracted repeatedly with ether. Combined extracts were dried over sodium sulphate and evaporated in vacuum. In special cases methyl esters were prepared by well known trans-esterification (0.4% sodium methoxide) or diazomethane procedure.

(v) Direct TLC. Analytical direct TLC of oils or methyl esters were carried out on a 20 cm x 5 cm plate coated with a 0.25 mm layer of silica gel G. The plate was developed with a mixture of ether-pet.ether-acetic acid. Visualization of the spot was made by spraying with sulphuric acid and subsequent charring.

(vi) Reversed-phase TLC. Reversed-phase TLC of the esters were done on siliconised silica gel G plate using acetonitrile-acetic acid - water (70:10:20; V/V) as developing solvent. Spraying of the chromatoplate was done with chromic acid.

(vii) Argentation TLC. Argentation TLC was effected on silica gel G plate impregnated with 12% silver nitrate. Solvent system ether - pet.ether (8:92; V/V) used for development. The spots were detected under u.v. light by spraying with 2', 7'-dichloro-fluorescein.

(viii) Infrared. I.r. spectra were recorded on liquids or carbon tetrachloride solution using Perkin-Elmer spectrophotometer (Model 221).

(ix) Ultraviolet. U.v. spectra of oils were recorded on SP 700 Unicam double beam spectrophotometer using methanolic solution.

(x) Gas-liquid chromatography. GLC of the methyl esters were carried out using F and M Model 720 GLC unit provided with thermal conductivity detectors using a 2' x 3/16" column of silicone (SE 30, 2%) and a 8' x 3/16" column of polyester (Diethylene Glycol Succinate, 15% on chromosorb W, 45-60 mesh). Temperature at the injection port, detector block and column were 290°, 260° and 200° respectively. Attenuation 4, bridge current 150 m. amp. and chart speed 15 inches/hr. Hydrogen flow rate 60 ml/min.

(A) Seed Oils of the family Apocynaceae.

Four seed oils belonging to different genera of Apocynaceae have been analysed for their total fatty acid composition. Out of the four oils the oil of Wrightia tomentosa on qualitative TLC revealed the presence of an unusual acid which appeared to be a major component of the mixed fatty acids. The other three oils (Thevetia neriifolia, Vallaris solanaceae, Plumeria acuminata) showed no indication of any unusual component.

Exhaustive extraction of the seeds of W. tomentosa with light petroleum gave an oil (yield 22%), which had the following characteristics: IV 93, HV 106, AV 6.1, n_D^{30} 1.4691. The i.r. (liquid film) and u.v. showed no indication of conjugation or trans or α,β-unsaturation. The i.r. spectrum did exhibit a wide, shallow hydroxy band at 3600-3300 cm^{-1}. These characteristics of

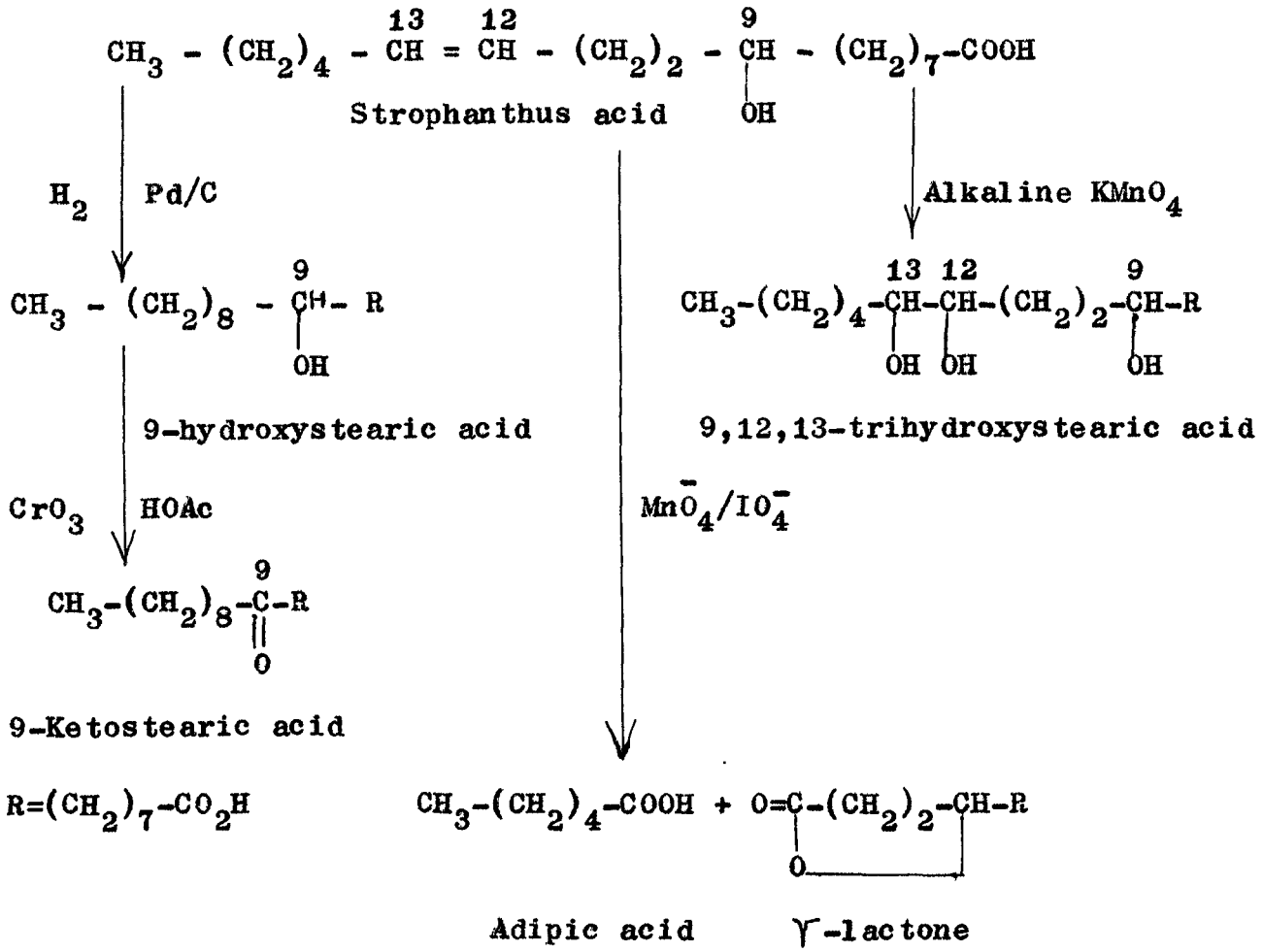
W. tomentosa oil suggested the existence of a hydroxy acid in the oil. Direct silica gel TLC of the oil along with castor oil showed a large spot moving a little slower than the major castor oil component (triricinolein). Direct TLC of the methyl esters (prepared from the oil by alkaline or acid catalysed methanolysis) showed a spot (Rf 0.21) similar to that of methyl ricinoleate (Rf 0.23), the other components moving with solvent front. Reversed-phase TLC (using acetonitrile-acetic acid-water) of the methyl esters showed small spots for linoleate, palmitate + oleate and stearate and a large oxygenated ester spot of Rf 0.50. Argentation TLC of the esters indicated the presence of only monoene and diene in the fatty acids. These observations so far made taken together pointed to the hydroxy acid being a C18 acid and with a structure similar to the hydroxy acid in castor oil. To determine the absolute identity of the Wrightia hydroxy acid it was isolated from the mixed acids by the method of Gunstone partitioning of freshly prepared fatty acids between 80% methanol and light petroleum. The polar acid obtained from the methanolic phase was esterified and acetylated in the usual way. The acid ester and its acetylated derivative after purification by column chromatography showed single spot on TLC and were thus homogeneous. Catalytic hydrogenation (pd/C) of the acid gave crystalline 9-hydroxy-octadecanoic acid, m.p. 81-82^{o} and of the ester the corresponding product methyl 9-hydroxyoctadecanoate, m.p. 52-54^{o}. Hydroxylation

of the acid by alkaline potassium permanganate following the procedure of Kass and Radlove[42] yielded two trihydroxystearic acids, melting at 108-110° and 147-148°. The saturated hydroxy acid on chromic acid oxidation yielded 9-ketostearic acid, m.p. 80-81°, the i.r. spectrum of which showed a clear band at 1710 cm^{-1} characteristic of free saturated carbonyl. All the melting points of the derivatives of the hydroxy acid correspond to that reported for strophanthus derivatives[10].

The positions of the double bond and of hydroxyl function in the fatty acid chain were established by the results of oxidative degradation. The isolated hydroxy ester was subjected to permanganate-periodate cleavage following the procedure of Von Rudloff[43]. The acidic fragments thus obtained were esterified by diazomethane and these examined by gas chromatography. The methylated product gave a strong absorption at 1772 cm^{-1} characteristic of a γ-lactone. GLC analysis using reference compounds showed the presence of methyl hexanoate and a component which could be C12 lactone methyl ester. All these data are consistent with the presence of double bond at C12 and of a hydroxyl group at C9 in the Wrightia fatty acid. Thus the hydroxy acid occurring in W. tomentosa oil is strophanthus acid, 9-hydroxy-cis-12-octadecenoic acid. The structure of this acid was further confirmed by direct comparison with a sample of pure strophanthus acid kindly

gifted by F.D. Gunstone. Direct co-chromatography gave a single spot. The i.r. spectra were superimposable and mixed melting points of some derivatives were undepressed.

The reactions carried out to establish the structure of Wrightia fatty acid as 9-hydroxyoctadec-12-enoic acid are given below.



Following GLC separation of silylated methyl esters on silicone and polyester columns, the amount of the component fatty

esters were calculated from the areas of recorded peaks. The weight percentages of the component acids: Palmitic, Stearic, Oleic, linoleic and strophanthus in the W. tomentosa oil lies close to 8.1, 3.8, 9.9, 11.24 and 67 respectively.

Till now the occurrence of strophanthus acid has been restricted to three genera of the family Apocynaceae, namely Strophanthus[11], Holorrhena and Nerium[12]. The occurrence of strophanthus acid in the genera Wrightia is now for the first time reported. Except H. antidysentrica seed oil[12] the seed fat of W. tomentosa is so far the richest known sources of strophanthus acid.

The other three seed oils of Apocynaceae (T. neriifolia, V. Solanaceae, P. acuminata) which were found to be usual oils, when analysed by GLC had the following fatty acid composition (Table - 1).

Table - 1.

Fatty acid composition of (T. neriifolia, V. solanaceae, P. acuminata) seed oils.

Name of plant	Acid Wt. %				
	16:0	18:0	18:1	18:2	20:0
Thevetia neriifolia	25.9	7.1	41.6	24.3	1.1
Vallaris solanaceae	12.5	11.1	22.0	54.3	-
Plumeria acuminata	32.7	1.6	23.0	42.7	-

The oil of T. neriifolia had already been analysed by Bhattacharya and coworkers[44] by classical methods. The present fatty acid composition is different from their findings.

EXPERIMENTAL

Thin-layer chromatography.

Direct TLC of the Wrightia oil along with castor oil using 40% ether in light petroleum showed a large spot moving a little slower than the major castor component (triricinolein). Direct TLC of the esters using 15% ether in light petroleum showed a spot (Rf 0.21) similar to that of ricinoleate (Rf 0.23), the other components moving with the solvent front. Argentation TLC using 8% ether in light petroleum of the esters yielded small spots for monoene and diene and a large slow moving spot similar to methyl ricinoleate. Reversed-phase TLC (siliconised) using acetonitrile-acetic acid-water (70:10:20; V/V) of the esters showed a large oxygenated-ester spot followed by small spots for linoleate, palmitate + oleate and stearate.

Isolation of hydroxy acid.

The freshly prepared mixed acids of each oil were immediately partitioned according to Gunstone[45] between light petroleum and 80% methanol. The hydroxy acid present in each alcoholic layer was collected, dried and after methylation purified by column chromatography using ether-petrol (5:95; V/V). The isolated ester gave a single spot by direct TLC.

Characterization of hydroxy acid.

(a) Hydrogenation of pure hydroxy acid and its methyl ester were done over palladium-carbon (10%) in ethyl acetate at 45 Psi at room temperature. Total hydrogenation of the acid for 2 hr gave a solid which on crystallization from benzene yielded pure saturated hydroxy acid melting at 80-81° (lit.[10] 9-hydroxystearic acid, m.p. 80-81.5°). Hydrogenation of hydroxy ester for 8 hr gave a product, which was crystallized from hexane, and melted at 52-53° (lit.[10] methyl 9-hydroxystearate m.p. 50-53°), depressed to 45-50° on mixing with 12-hydroxystearate (m.p. 58-59°) derived from hydrogenation of methyl ricinoleate.

(b) Oxidation. A 1 g portion of the saturated hydroxy acid was oxidized with chromium trioxide (3.2 g) in acetic acid following the procedure of Smith and coworkers[14]. The reaction product was crystallized twice from methanol to yield 0.55 g of a keto acid melting at 80-81° (lit.[10] 9-ketostearic acid, m.p. 79.5-81°), i.r. 1710 cm^{-1} (carbonyl).

A solution of unsaturated hydroxy acid (5 g in 2500 ml water) containing 1.6 g of KOH was hydroxylated with $KMnO_4$ (5.3 g) dissolved in 250 ml water. The oxidation product when worked up according to the procedure of Kass and Radlove[42] gave two trihydroxystearic acids, one chloroform soluble, m.p. 108-110° (identical with literature for 9, 12, 13-trihydroxystearic acid)

and the other chloroform insoluble, m.p. 147-48° (lit.[10] m.p. 148-49°).

(c) Oxidative degradation. The unsaturated hydroxy ester was cleaved oxidatively by Von-Rudloff's reagent[43]. The GLC analysis of the methylated fragments showed the presence of methyl hexanoate.

GLC analysis of the mixed esters of W. tomentosa seed fat as silylated methyl esters (with silicone and polyester columns) showed the following composition (Table - 2), as percentage weight esters.

Table - 2.

GLC analysis of silylated methyl esters of W. tomentosa.

Type of acids	On silicone column
C16	6.5
C18	23.7
Strophanthus	69.8
	On polyester column
Palmitic	10.7
Stearic	4.3
Oleic	10.9
Linoleic	12.8
Strophanthus	61.3

(B) Seed Oils of the family Malvaceae and Sterculiaceae.

During the last decade the application of modern methods of analysis has shown that the occurrence of cyclopropenoid acids in seed oils is not as uncommon as once believed. Cyclopropenoid fatty acids have been found in some seed fats of the Malvaceae, Sterculiaceae, Tiliaceae and Bombacaceae families[46,47]. Though the two cyclopropenoid acids, malvalic and sterculic often occur together, but the seed oils of the Malvaceae tend to contain more malvalic, and those of Sterculiaceae mostly sterculic. The seed oil of Sterculia foetida is unusually rich (50%) in sterculic acid, accompanied by small portions (5%) of malvalic acid.

In the present study two seed oils of Altheae officinalis (Malvaceae) and of Pentapetes phoenicea, (Sterculiaceae) have been analysed for their fatty acid composition by TLC-GLC techniques. Sterculia foetida oil esters were used for reference wherever needed. The method of hydrogen bromide titration[41] was followed to estimate the cyclopropenoid acid content.

Light petroleum extraction of the seeds gave 12% oil in A. officinalis and 13.5% oil in P. phoenicea. Both the oils responded to Halphen's[48] test thereby indicating the presence of cyclopropenoid acid. The i.r. and n.m.r. spectra of the oils showed absorption at 1010 cm^{-1} and signals at 9.28τ characteristics of cyclopropenoid group. The u.v. spectra showed no conjuga-

tion in the oils.

The esters of each oil obtained by base-catalysed transesterification were examined qualitatively by direct, reversed-phase and argentation TLC along with S. foetida esters serving as the cyclopropenoid acid reference. Direct TLC showed no oxygenated acid. The reversed-phase TLC showed a spot near the starting point as exhibited by S. foetida esters. Argentation TLC showed the expected spots of saturates, monoene and diene. An extra spot was visible just below the saturates which must have been derived from cyclopropenoid acid present in the oil.

GLC analysis of methyl esters.

The identification and qualitative estimation of the cyclopropenoid acid present in A. officinalis and P. phoenicea seed oils was made by GLC of esters as such and after hydrogenation, using both silicone and polyester columns. Identification of peaks of malvalic and sterculic acid esters was made by comparison with those of reference esters of S. foetida oil.

Since it is known that malvalic acid peak is masked by linoleic acid peak, GLC analysis of A. officinalis esters on both the columns did not show a complete separation of malvalic acid ester peak. To circumvent this difficulty the GLC analysis of the hydrogenated esters was done on polyester column. A peak

emerged after C18 peak and had an ECL of 18.3. Hydrogenated esters of S. foetida on the same column showed a small peak on the same position. A comparison of the observed ECL value with that of the reported[46] ECL value (Dihydromalvalic: Polyethylene glycol adipate 18.3) showed the presence of malvalic acid in the total fatty acids.

GLC analysis of P. phoenicea esters before and after hydrogenation clearly showed the characteristic peaks of sterculate (ECL 19.9) and of dihydrosterculate (ECL 19.4). A comparison of the observed ECL values with those of the reported ECL values (Sterculic acid: Apiezon-L 18.5; polyethylene glycol adipate 19.9) established the presence of sterculic acid in the total fatty acids. Further the observed dihydrosterculic ester ECL values (silicone 18.8 and polyester 19.4) are in agreement with the values (Apiezon-L 18.8; Resoflex 19.4) reported by Smith and coworkers[49].

The close similarity of equivalent chain lengths confirmed the inference obtained from the results of Halphen test, TLC and hydrogen bromide titration. Thus A. officinalis (Malvaceae) and P. phoenicea (sterculiaceae) seed oils contain malvalic and sterculic acids respectively.

The GLC analysis of the seed oils indicated the following fatty acid composition (% Wt. acids):

A. officinalis: Palmitic, 16%; 8-(9-)methyl heptadecanoic, 3.0%; Stearic, 2.30%; linoleic, 44.5%; malvalic, 11.7%.

P. phoenicea: Palmitic, 25.61%; Stearic, 1.80%; Oleic, 22.20%; linoleic, 47.20%; Sterculic, 3.4%.

EXPERIMENTAL

Coarsely ground seeds (250 g) of A. officinalis and P. phoenicea were extracted with petroleum ether (40-60) in cold overnight. Removal of the solvent under reduced pressure in a current of nitrogen yielded 12% and 13.5% of the oils respectively showing the following characteristics:

A. officinalis n_{29}^{D}, 1.4689; I.V., 112; A.V., 6.5.

P. phoenicea n_{29}^{D}, 1.4696; I.V., 108; A.V. 3.9.

I.R. of the oils in CCl_4 showed characteristic absorption for cyclopropene moiety at 1010 cm^{-1} and n.m.r. peak at 9.28 τ.

Halphen color reaction.

A solution of sulphur (1% in carbon disulphide) was prepared for the Halphen test. Oil (1 ml) was taken in amyl alcohol (1 ml) and mixed with 1 ml of the above reagent. The mixture was heated on a water bath for a few minutes till CS_2

had boiled off. On keeping the test tube in an oil bath (110-115°) for 1-2 hours, a red color characteristic of cyclopropenoid acid developed. The color for A. officinalis oil came earlier and was more intense.

Determination of cyclopropenoid acid content.

Hydrochloric acid treated oil (2.3 g) was weighed into a flask to which was added 5 ml of benzene and 15 ml of acetic acid. The mixture was maintained at 3°, and titrated with Durbetaki reagent[50] (HBr in glacial acetic acid, 0.1N) to a blue-green end point using crystal violet as indicator. This gives the epoxy acid contents, if any, but none was present. The temperature was then raised to 55° and the solution was titrated again to a blue-green end point.

The percentage of cyclopropenoid acid was calculated by the equation:

$$\% = \frac{29.45 \times H \times V}{W}$$

A. officinalis : 11.7%

P. phoenicea: 3.4%

Preparation of methyl esters.

The fatty acid methyl esters were prepared by trans-

methylation of 2 g of the oils in 50 ml of methanol containing 1% sodium methoxide as catalyst. The reaction was allowed to proceed at room temperature with stirring for 24 hr. The methanol was removed under reduced pressure and the residue was diluted with water and extracted with ether. The extracts were washed free of the alkaline catalyst with distilled water, dried over anhydrous sodium sulphate and the solvent was evaporated in a stream of nitrogen to yield 1.8 g of methyl esters.

Hydrogenation of esters.

The esters (1.2 g) taken in 50 ml methanol were hydrogenated with palladium-carbon (0.4 g) in a Parr-low pressure-hydrogenator at 40-45 psi for 8 hours. Esters were recovered as usual.

Direct TLC of esters.

A glass micro-chromatoplate (2.5 x 7.5 cm) coated with silica gel-G was spotted with the methyl ester. Development with 15% ether in petrol and subsequent charring with chromic acid gave only one spot near the top of the chromatogram.

Reversed-phase TLC.

The hydrogenated and non-hydrogenated esters were chromatographed on a silica gel-G plate impregnated with silicone oil,

S. foetida esters (as such and hydrogenated) were also spotted on the same chromatoplate as reference compounds. Development of the siliconized plate in the solvent system, acetonitrile-acetic acid-water (70:10:20; V/V) and subsequent spraying with chromic acid showed in all the three esters clear spots of the usual critical pairs, and a spot near the base line corresponding to cyclopropenoid ester.

Argentation TLC.

Methyl esters of the oil were spotted on a 20 x 5 cm plate of silica gel-G impregnated with silver nitrate (12.5%). The chromatoplate was developed with ether in petroleum ether (8:92; V/V). Spraying with 2',7'-dichlorofluorescein and viewing under u.v. revealed spots of saturates near the front followed by cyclopropenoid ester, monoene and diene, parallel to those obtained from S. foetida esters resolved alongside.

GLC of methyl esters.

Identification of component acids and their quantitation was carried out using GLC analysis by comparison with esters (both non-hydrogenated and hydrogenated) of the oil of S. foetida. GLC runs were made both on silicone and polyester columns. The calculated fatty acid composition and the equivalent chain length (ECL)

values are given below:

Fatty acid composition of A. officinalis

Silicone

	Ester	Hydrogenated ester	ECL
C16	16.0	15.85	16.0
8-(9-)methyl-heptadecanoic	-	2.81	17.17
C18 + Malvalic	84.0	84.10	18.0

Polyester

	Ester	ECL
Palmitic	16.0	16.0
8-(9-)methylheptadecanoic	3.0	17.15
Stearic	2.30	17.8
Oleic	22.30	18.2
Linoleic + Malvalic	56.20	18.8

Polyester

	Hydrogenated ester	ECL
C16	15.80	16.0
8-(9-)methylheptadecanoic acid	2.81	17.18
C18	69.30	18.0
Dihydromalvalic	11.70	18.3

Fatty Acid Composition of P. phoenicea

Silicone

	Ester	Hydrogenated ester	ECL
C16	25.42	25.53	16.0
C18	71.20	71.23	18.0
Sterculic	3.41	-	18.5
Dihydrosterculic	-	3.10	18.8

Polyester

	Ester	ECL
Palmitic	25.61	16.0
Stearic	1.80	18.0
Oleic	22.20	18.8
Linoleic	47.20	19.2
Sterculic	3.40	19.9

Polyester

	Hydrogenated ester	ECL
C16	25.39	16.0
C18	71.30	18.0
Dihydrosterculic	3.38	19.4

(C) Seed Oils of Other Families.

The extracted oils from the seeds of the species mentioned in Table - 3 were analysed by u.v., i.r. and combined TLC and GLC. The u.v. spectra did not show the presence of conjugation in the oils. The i.r. spectra gave no information for the presence of trans-unsaturation or any unusual functional group. The methyl esters of mixed fatty acids obtained from each oil by the usual procedure were subjected to preliminary analysis by using various forms of thin-layer chromatography. Direct TLC revealed the presence of only non-oxygenated acids. In reversed-phase TLC using a siliconised silica gel-G plates the spotting of saturated methyl esters gave distinct spots only for palmitate and stearate. Argentation TLC of the methyl esters using silver nitrate impregnated silica gel-G plate revealed spots corresponding to the saturated, monoene, and diene esters. Absence of spot for triene indicated that none of the oils (except Gmelina arborea) contain the common C18 triene, linolenic acid. The methyl esters of each oil were run on GLC and quantitation of the fatty acids was made using triangulation method. The results of the qualitative, direct, reversed-phase and argentation TLC supported the findings of GLC analysis. The fatty acid composition (wt %) of the various seed fats are given in Table - 3. Some comments are given below:

The oil of Hyoscyamus niger (Solanaceae) was first analysed by Lutenbirg and coworker[51], who reported the composition to be

palmitic + stearic (10%), oleic (16%) and linoleic (74%). In the oil from the Indian variety of the seed there is found no marked difference in the fatty acid composition. The seed oil of 5 species of Coniferae have been analysed which were found to contain predominantly oleic and linoleic acids as major components. These two acids constitute more than 80% of the total fatty acids of these oils. The oils of the Jatropha gossypifolia (Euphorbiaceae), Cleome viscosa (Capparidaceae), Basella rubra (Chenopodiaceae), were found to contain the common acids, palmitic, stearic, oleic and linoleic. Two oils of the family Verbenaceae, Gamelina arborea and Vitex negundu were found to differ markedly in their fatty acid composition. Besides the usual fatty acids mentioned above G. arborea seed oil contains the triene acid, (linolenic, 7.2%) and C20 saturated (arachidic, 1.2%). These minor acids were found to be absent in the oil of V. negundu. Two other oils of Lactuca scariola (Compositae) and Nyctanthus arbor-tristis (Oleaceae) were also found to contain traces of arachidic acid. All the oils reported in Table - 3 belong to mainly palmitic-stearic-oleic-linoleic type of usual oils. The oils of H. niger, P. strobus, P. echinata and J. gossypifolia belong to the class of drying oils. Due to their unusually high content of linoleic acid ($>$60%), they may in future be used in paints and allied industries.

Table - 3.

Fatty acid composition of Seed oils

Name of plant	Family	Iodine value	Acid wt. %							
			12:0	14:0	16:0	18:0	18:1	18:2	20:0	18:3
Hyoscyamus niger, Linn.	Solanaceae	138	-	-	13.6	3.7	11.6	71.1	-	-
Lactuca scariola, Linn.	Compositae	133	-	-	8.6	1.6	30.1	59.1	0.6	-
Nyctanthus arbortristis	Oleaceae	91	-	-	17.1	2.2	68.9	10.2	0.8	0.8
Basella rubra	Chenopodiaceae	81	-	-	20.9	5.8	54.3	18.2	0.8	-
Gamelina arborea, Linn.	Verbenaceae	103	-	0.2	14.7	5.9	48.2	22.6	1.2	7.2
Vitex negundu	,,	119	-	-	9.5	8.6	30.7	51.2	-	-
Cleome viscosa, Linn.	Capparidaceae	117	-	0.3	15.1	7.5	23.5	53.6	-	-
Pinus strobus	Coniferae	139	0.1	-	6.7	0.6	29.8	62.8	-	-
Abies pindrow	,,	118	1.8	2.3	3.1	0.2	54.2	38.4	-	-
Pinus fusularis	,,	126	-	-	10.7	1.1	35.6	52.6	-	-
Pinus ekioti	,,	112	2.2	-	10.3	14.5	41.5	41.5	-	-
Pinus echinata	,,	139	-	-	6.1	1.2	30.1	62.6	-	-
Jatropha gossypi folia	Euphorbiaceae	137	-	0.4	13.3	3.4	13.4	69.5	-	-

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P A R T - II

Oxygenated Fatty Acids

Prior to the middle of 1940's studies on natural fats were limited to the study of fatty acid composition of seed oils. The lack of academic interest in the study of chemistry of fatty acids may be attributed to their not being readily crystallizable materials and their complexity inherent in their structures. Another limiting factor responsible for a slow progress in this field was the impression that fatty acids exist in solution in associated dimeric state and crystallize as such from solutions. Among natural products the higher fatty acids molecules are structurally unique in possessing a long non-polar hydrocarbon chain which terminates in highly polar carboxylic group. It is this unbalanced polarity which accounts for their very valuable properties.

The growing importance of fatty acids in technology has stimulated research in fatty acid oxidation, a field which has been most extensively investigated during the last two decades. The oxidation of fatty acids has been studied under three heads depending upon the nature of products: (a) Hydroxylation, (b) epoxidation and (c) oxidative degradation. There are quite a few oxidizing agents and so are the products derived from their

action. These products more often are called "Oxygenated fatty acids" possess the remarkable property of being high-melting and easily crystallizable as compared to their non-oxygenated analogues. Oxygenated fatty acids have not achieved the industrial importance of non-oxygenated acids, mainly because of their nonavailability from natural sources or synthetically. These acids are now considered to be essential intermediates in a variety of basic studies in the fatty acid chemistry, i.e. autoxidation, chemical oxidation and the reaction mechanism and the metabolic investigations. Oxygenated acids are also attractive compounds for studies of positional, geometric and optical isomerism and for studies of the effect of, type and number of functional groups on their physical and chemical properties.

The work on the oxygenated fatty acids has been centred mainly on three lines:

(i) Preparation of various types of oxygenated acids from unsaturated fatty acids.

(ii) Interconversion of oxygenated acids.

(iii) Mechanism and stereochemistry of the reactions that occur in the preparation and mutual transformation of oxygenated fatty acids derivatives.

A review of the literature on the formation and reactions of oxygenated fatty acids indicates that the divergent results

reported from time to time are mainly due to the difficulties experienced in controlling the course of the reaction and in selecting the criteria of purity. It thus appears that there are a number of factors which influence the course of fatty acid reactions.

(i) The first factor is the possibility of the formation of primary and secondary oxidation products which can be isolated by the use of a single oxidant.

(ii) The second aspect of the oxidation reaction is the stability of the oxygenated acids formed during oxidation. It has been observed that reactions of a competitive character lead to the formation of stable as well as unstable oxidation products. In some cases the oxidation products are readily oxidizable than the starting material.

(iii) The third factor is the influence of the nature of the solvent, the action of acid or base in catalyzing the reaction and the role of temperature on the oxidation process.

(iv) Lastly the steric effect of the substituents attached to the ethylenic carbon atom or the spatial effect of the groups may also govern the nature of oxidation reaction and lead to the formation of unexpected reaction products.

In spite of the use of pure fatty acids and known specific

oxidants, the available methods of isolation and characterization have not been adequate in determining the mechanism of the formation of oxygenated fatty acids and in explaining the exact nature of the intermediates and the end products.

During the first quarter of present century very little was known about the stereospecificity of organic reactions of double bonds. With the growing understanding of the mechanism of organic reactions the controvercial problems of organic chemistry were gradually solved. It is rightly pointed out that the different phases of the development and progress of organic chemistry are better exemplified by the general perfection achieved by the chemistry of oxygenated fatty acids.

The large variety of products resulting from the oxidation of a fatty acid has been the main drawback in its systematic study. The opportunities which exist require that extreme care should be taken in their preparation, isolation and in selecting criteria of purity. A survey of the early literature reveals that the results obtained in different type of oxidation studies have led to the interpretations which are conflicting as far as the mechanism and stereochemistry are concerned. The study on the course of configurational changes involved in glycol formation via the intermediate halohydrins and oxiranes have been made by a number of workers[1-7]. The confusion prevalent in such studies was the result of divergent experimental data reported by different

investigators from time to time. This was due to interpretations made on the basis of not well understood conceptions of theories of organic reactions. It is now realized that organic chemistry is, to a large extent, the study of reactions of functional groups with important contributions of polar, steric, conformational and neighbouring group effects. The realization of these factors in the third quarter of present century has led to a renaissance in the chemistry of fatty acids.

The generalizations of Swern[3] and Gunstone[8] on the stereochemistry of fatty acids reactions have been responsible for a clear understanding of the chemistry of oxygenated fatty acids. Swern proposed a reaction scheme which correlates the configurational relationship in the conversion of oleic and elaidic acids to their corresponding glycols via their halohydrins and epoxides. A systematic study of the oxidation and hypohalogenation of petroselenic and petroselaidic acids carried out by Farooq and coworkers[6-10] and the work of Roomi et al.[7] on erucic and brassidic acids have given further experimental evidences in favour of Swern's reaction scheme.

Epoxy fatty acids represent one of the important classes of oxygenated acids. They contain the most versatile, reactive and interesting functional group 'oxirane', a three membered heterocyclic ring. The commercial use of epoxidized oils and esters in recent years has highlighted the interest of fat chemist

in the study of oxirane compounds. Oxiranes react in solution with a wide variety of electrophilic, neucleophilic and neutral reagents. Although lability of epoxides in the presence of an enormous variety of reagents has been known and exploited for many years, much confusion has surrounded the mechanism of these reactions. The direction and stereochemistry of ring opening are governed largely by three factors (a) structure of epoxide; (b) structure of reagent; (c) reaction conditions, such as temperature, solvent polarity and catalyst.

During the last decade new and interesting reactions of oxygenated fatty acids have been described that provide new route to the synthesis of a variety of fatty compounds. The growing demand of fatty chemicals as intermediate raw materials has diverted the attention of fat chemist from the analytical aspect of natural fat to the theoretical side of the reactions of unusual fatty acids and their novel derivatives. Recent advances in chromatographic methods of separation and spectroscopic methods of structure determination have made it possible that complex reactions of polyfunctional fatty acids can now be examined more profitably. This has led to the work on the kinetics of reactions and the use of physico-chemical data in solving the mechanistic problems of oxygenated acids. Thus the conventional element data, melting point determination and derivatization of reaction products are no longer the sole criteria for structure determination. The

spectral methods of characterization is now considered more reliable than the earlier methods of investigation. Among the various spectroscopic disciplines the use of n.m.r. and mass in the study of fatty acid reactions has attracted considerable attention in recent years. Therefore it is considered desirable to give a short account of the applications of n.m.r. and mass spectral studies in the fatty acid chemistry.

Nuclear Magnetic Resonance.

NMR was discovered quite early but its use in fatty acids and related compounds is of recent origin. A number of reviews[11-13] on the n.m.r. spectra of fatty acids have appeared in the literature. NMR has been particularly more useful in the structure determination of unusual functions in the fatty acid chain and in characterizing the products of their reactions. In straight chain saturated fatty acids, there are essentially four peaks in the spectra. These represent the terminal methyl group protons centred at 9.1τ, the α-CH_2 protons centred at 7.7τ, the remaining isolated methylene protons centred at 8.7τ, and the acid proton at low field. The absence of a peak near 9.1τ does not contraindicate a terminal methyl group. Methyl protons of the ester group give a single sharp peak at 6.3τ. This peak is useful as a reference for area measurement. In olefinic acid,

when the double bond is near the centre, the vinylic protons give signals at 4.56τ and α-methylene to double bond at 8.0τ. Methylene group β to double bond give signal at a slightly low field than the isolated one. The terminal double bond in 10-undecenoic acid gives separate signals for the CH_2=(5.1τ) and for =CH protons (4.3τ).

Current interest in unusual fatty acids indicates the value of additional data on spectra-structure correlations. Particular interest is focused on the changes in n.m.r. spectra produced by cis- trans isomerism and by conjugation. The highly complex spin systems formed by these molecules often give rise to broad unresolved or partially resolved multiplets which result from a large number of closely spaced transitions. The broad multiplets are useful as finger print patterns and permit distinction among similar structural groupings involving multiple C=C bonding. In the case of oxygenated acids, hydroxy acids give rise to two peaks in n.m.r. which represent the O-H proton and -CH-O- proton. This methine proton appears at 6.4τ, while the signal of the proton of hydroxyl group changes with dilution and can be eliminated by a drop of D_2O. In an acetoxy derivative the acetyl protons produce a sharp singlet at 7.9τ. The epoxy ring protons appear at 7.2τ. This peak coincides with diallylic CH_2 peak (7.2τ) but the epoxy group can be converted to diacetoxy which gives a new peak at 7.9τ. Therefore the presence of

epoxy or hydroxy functions can be confirmed by acetolysis or acetylation and examining the n.m.r. spectra.

In the current literature there are a large number of examples of the application of n.m.r. in solving the structural problems of fatty acids. Gunstone and Ismail[14] and Purcell et al.[15] have recently studied the n.m.r. spectra of isomeric C18 fatty acids. Acids with unsaturation near the centre of the molecule cannot be distinguished from one another. But the isomers with unsaturation closest to either the carboxyl or the end methyl group show distinctive spectral features which allow them to be identified. There is also a distinctive difference in the coupling forces acting on olefinic protons depending on whether the configuration is cis or trans, especially in α,β-unsaturated acids. J values for trans olefinic protons[16] are regularly higher than those corresponding to cis protons. Fatty acids with trans α,β-double bond have a coupling constant $\sim$16 c/s. Barker et al.[17] gave values for methyl 2-undecenoate as J cis 11.3 and J trans 15.7 c/s.

An example of the application of various techniques developed to extend the value of n.m.r. may here be cited from the literature. Tallent and coworkers[18] with the help of spin decoupling technique established the structure of coriolic acid as 13-hydroxy-9-cis, 11-trans-octadecadienoic acid. NMR spectroscopy is a field undergoing rapid development. It is expected that new

application of n.m.r. in the field of fat chemistry will result from the improvements in the technique and a better understanding of its theoretical basis.

Mass spectrometry.

The mass spectrometric fragmentations of many simple lipids have been studied extensively mainly because they are sufficiently volatile and stable to be examined. In the field of fats the esters of higher fatty acids have been investigated thoroughly and indeed long chain fatty esters are one of the earliest and most widely examined classes of natural products. Their simple molecular structure and clear pattern of fragmentation make the interpretation of their mass spectra an easy affair. A number of reviews[19-24] on the mass spectrometry of fatty acids have been written in recent years.

Applications of mass spectrometry to the chemistry of fatty acids may be classified as to its use in (a) structural determination, (b) qualitative identification and (c) quantitative analysis. Usually two or three of these elements are present in any given application. In many fatty materials of interest, parent peaks are present and immediately provide the investigator the molecular weight of the material. As the fragmentation pattern observed is characteristic of the molecule broken, the diagnostic

ions are used for finger print type of empirical comparison and identification of compounds.

The parent peak of a straight chain fatty acid is usually weak but detectable. In long chain acids spectrum consists of two series of peaks resulting from cleavage at each carbon-carbon bond with retention of charge on either (a) oxygen-containing fragment (m/e 45, 59, 73, 87 - - -) or (b) alkyl fragment (m/e 29, 43, 57, 71, 85 - - -). The parent peak of a methyl ester of straight chain fatty acid is usually distinct. But it is weak in the range 120-200 but becomes more intense beyond this range. In general the fragmentation pattern for methyl esters approximates to that for fatty acids.

The mass spectrum of a straight chain fatty acid or its methyl ester shows a number of peaks due to ionized fragments that contain an intact-ester group. The ions responsible for these peaks are partly the result of elimination processes.

If the chain is branched, ionized fragments resulting from the breaking of the bond to tertiary or quaternary carbon atoms usually give rise to large peaks in the mass spectrum. From the mass over charge ratio (m/e) of these peaks the position of the side chain can, as a rule, be deduced. Mass spectrometry is the most powerful single method at present available for the structure determination of branched chain fatty acids.

In the case of esters $R_1 - \overset{O}{\overset{\|}{C}} - OR_2$ it is observed that peaks due to R_1^+, $(R_1CO)^+$, $(COOR_2)^+$, $(OR_2)^+$ and R_2^+ were detected for some of the esters of higher fatty acids. Without the aid of accurate mass measurement and high resolution, these ions can easily be confused with those of hydrocarbons, and the presence of oxygen atom cannot be detected directly. It is usually very difficult to distinguish the ion R_1^+ formed from an ester from the corresponding ion in the hydrocarbon spectra. The problem of rearranged peaks is also met within the spectra of fatty acids and their esters. The main advantage of high resolution in the examination of an ester lies in the fact that any such compound submitted for qualitative identification can immediately be recognized as containing two oxygen atoms. Fragment ions in the mass series 59, 73, 87, 101, etc. can contain two oxygens, as can the rearrangement ions at masses 47, 61, 75, 89 etc.

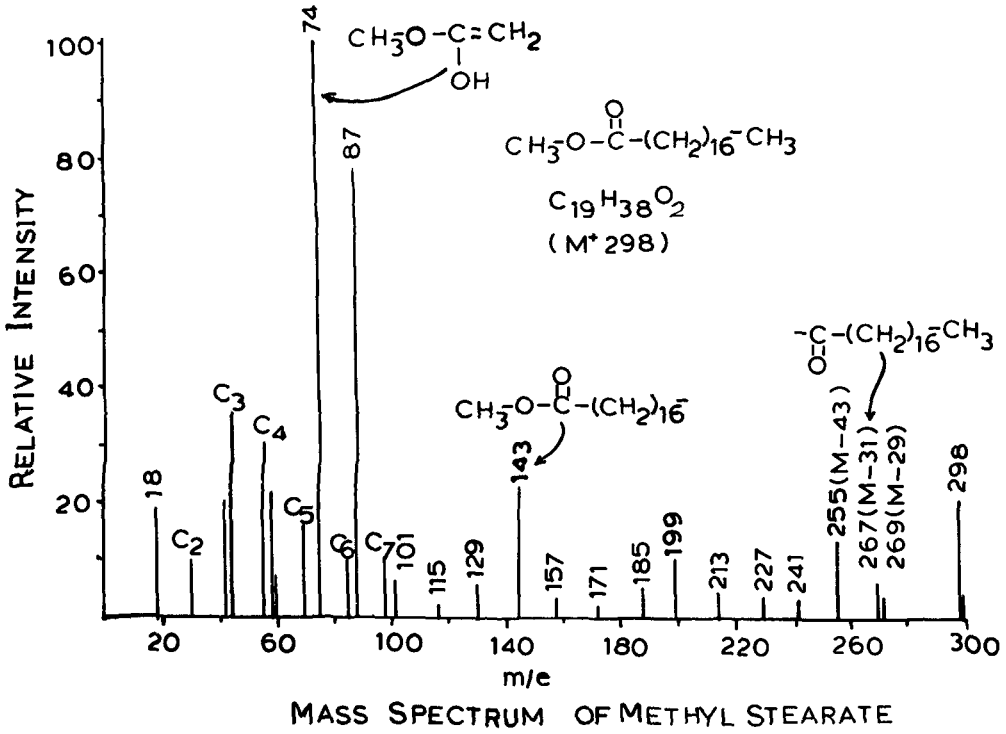
Hydrogen migration and skeletal rearrangement occur to a certain extent in a long-chain fatty ester molecule during mass spectrometric fragmentation. The ions of structure $(CH_2)_nCO_2CH_3^+$ can be formed either by simple cleavage of a carbon-carbon bond of the ester molecule, or by expulsion of a part of the chain plus a hydrogen atom, or by cleavage after single or multiple hydrogen shifts occurring in the molecular ion. Therefore, the hydrogen and carbon atoms in the resulting fragments may not be in the same positions in which they were in the original ester

molecule. Which mechanism dominates, and to what extent a mechanism is responsible for the formation of a $(CH_2)_nCO_2CH_3^+$ ion depends on which ion is under consideration. Much work done on the mechanism of mass spectrometric fragmentation of lipid molecules is mainly by substitution or isotope labelling at the site of interest in the molecule. The usefulness of deuterium-labelling of organic molecules for gaining insight into fragmentation and specifically rearranged processes under electronic impact have been demonstrated frequently in the mass spectrometry of fatty acids.

Esters of saturated fatty acids were among the first organic compounds whose mass spectra were studied in detail. The investigations of the pattern and mechanism of mass spectrometric fragmentation of fatty acid esters were first initiated by Stenhagen and Ryhage[21]. Their work provided considerable impetus to the application of this physical analytical method to other organic compounds. In the spectra the hydrocarbon ions results from the breakdown of the hydrocarbon chain of the molecule. These ions are of little diagnostic value. On the other hand the prominent peaks of the oxygen containing ions include the molecular ion peak (M^+), the acylium ion formed by loss of a methoxy group (M-31), the peak m/e 74 which is characteristic of most methyl esters, and a series of peaks of ions corresponding to $(CH_2)_nCOOCH_3^+$.

Spectra of saturated fatty acid esters.

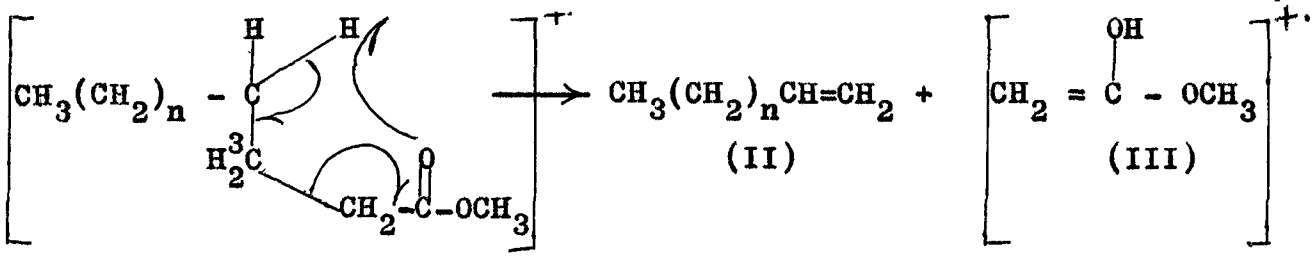
In the spectrum of methyl stearate (given below) the molecular ion peak is intense. It was found that in comparison with the base peak the intensity of the molecular peak tends to increase with increasing chain length of long chain esters[24,25].



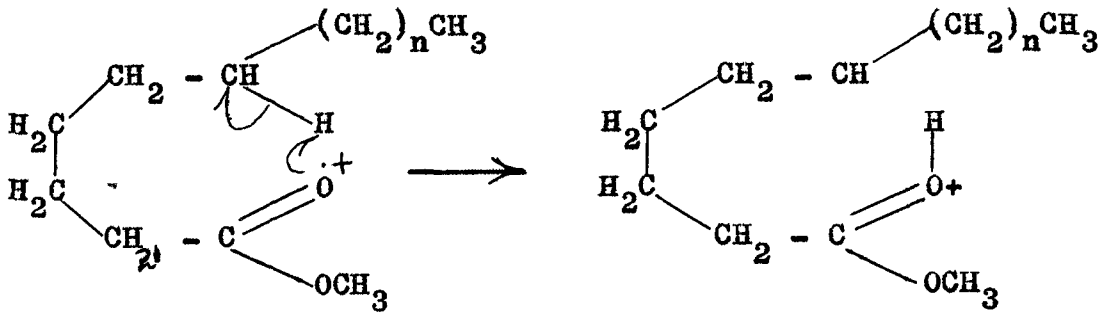
MASS SPECTRUM OF METHYL STEARATE

The base peak (m/e 74) results from a Mclafferty rearrangement, which transfers a γ-Hydrogen atom of the acid moiety to the carbonyl oxygen through a cyclic transition state (I) and cleaves

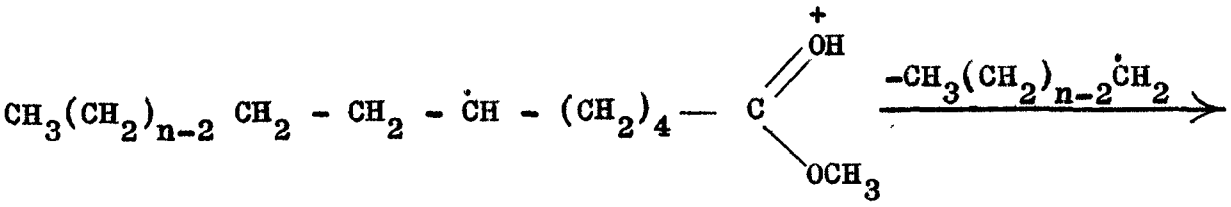
the C2-C3 bond to give olefin (II) and ion (III).

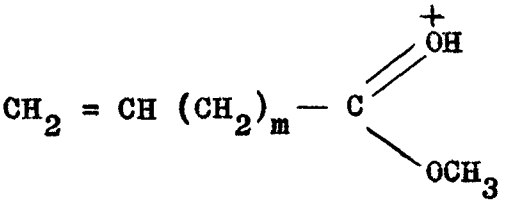


(I) (II) (III)



(IV) (V)





Between the peak m/e 74 and the molecular ion peak a series of peaks corresponding to the homologous ions $(CH_2)_nCO_2CH_3^+$ (n=2,6,10, 14; m/e 87, 143, 199 and 255 respectively) are observed. The peak m/e 87 is found to undergo hydrogen exchange with the methylene

group at position 5 or 6. Spiteller et al.[26] suggested that these ions possess the structure $CH_2 = CH - (CH_2)_m C^{+}(OH)OCH_3$ and are formed from the common intermediate (V), which results from a transfer of hydrogen atom from position 6 to the ionized carbonyl oxygen of the molecular ion (IV). Such type of rearrangements and bond ruptures occurring at different positions farther from the ester group give the above fragment ions. Two or more hydrogen shifts from the same intermediate (V) would explain the formation of the homologous fragment ions. By C^{13} or deuterium labelling experiments it has been shown that the peak at M-43 in the spectra of fatty esters results by an expulsion reaction which involves a loss of a propyl radical from the molecular ion to form a fragment of the type $(CH_2)_n COOCH_3^+$.

Spectra of unsaturated fatty acid esters.

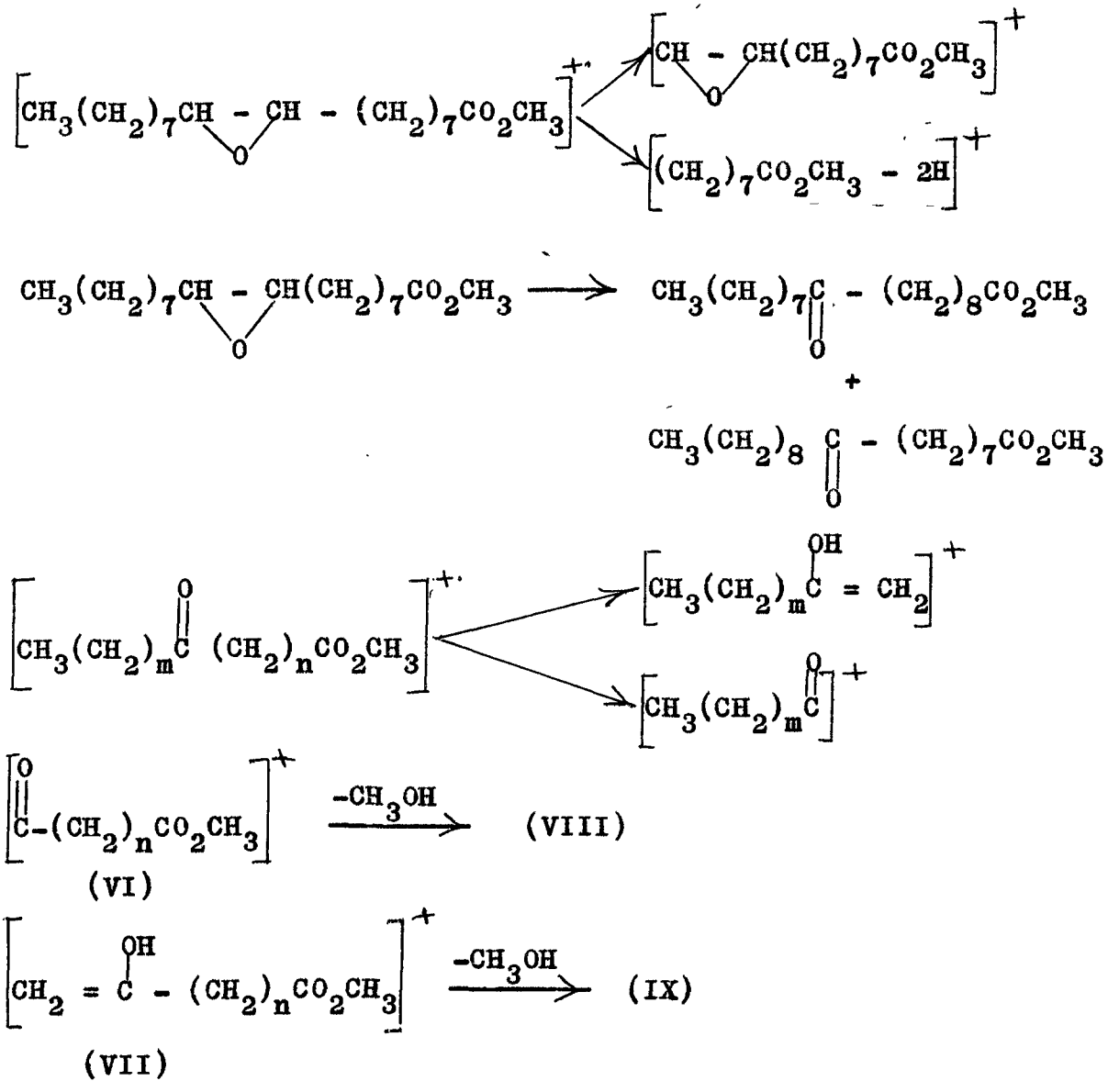
The spectra of unsaturated esters are complicated and give very little information about the molecular structure. Isomeric unsaturated esters have similar spectra except for the α,β-unsaturated esters. The spectra of methyl esters of monounsaturated isomeric acids (Oleic $\triangle^9$-cis-octadecenoic; elaidic, $\triangle^9$-trans-octadecenoic; petroselenic, $\triangle^6$-cis-octadecenoic) are almost identical indicating that neither positional nor geometrical

isomerism have the significant effect upon the fragmentation.

Attempts have been made in recent years to make use of mass spectrometry in the location of the position of double bonds in olefinic acids. The methods are based on the preparation of suitable derivatives of an unsaturated ester and then subject them to mass spectrometry. However, the application of these methods is restricted to monounsaturated fatty acids. The earliest approach to this problem was to saturate the double bond with deuterium with a view to obtain deuterium containing ions[27]. The method was not found to be helpful because of the complication caused by hydrogen and skeletal rearrangements. Another method to locate double bond was to convert an unsaturated ester to a cyclopropane derivative through carbene addition. Subsequently catalytic hydrogenation of the ring yields a mixture of two isomeric branched chain esters, the position of which can be determined by mass spectrometry[28].

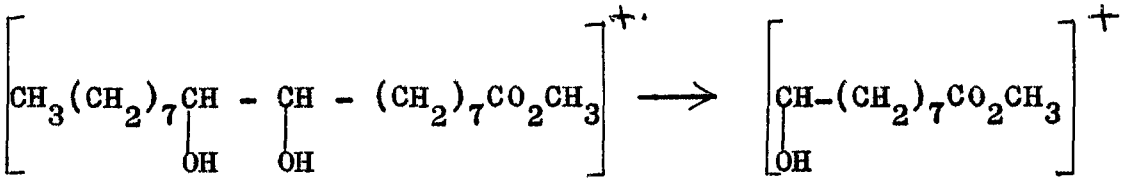
A more recent method developed was to oxidize a double bond to epoxide. The epoxide ring is isomerized to a mixture of two isomeric keto esters by use of sodium iodide. Six prominent peaks in the spectrum of each keto ester help to indicate the position of the keto group. Two peaks result from the cleavage of bond at each side of the keto group (α-cleavage) and two are formed by McLafferty rearrangement occurring at each side of the keto group. The remaining two peaks correspond to the loss of

methanol from the ions (VI) and (VII) to yield the secondary fragment ions (VIII) & (IX). Wolff and coworkers[29] have suggested the following fragmentation pattern for the above ions.



Audier and coworkers[30] reported that the position of the double bond in a long chain unsaturated compounds can be located

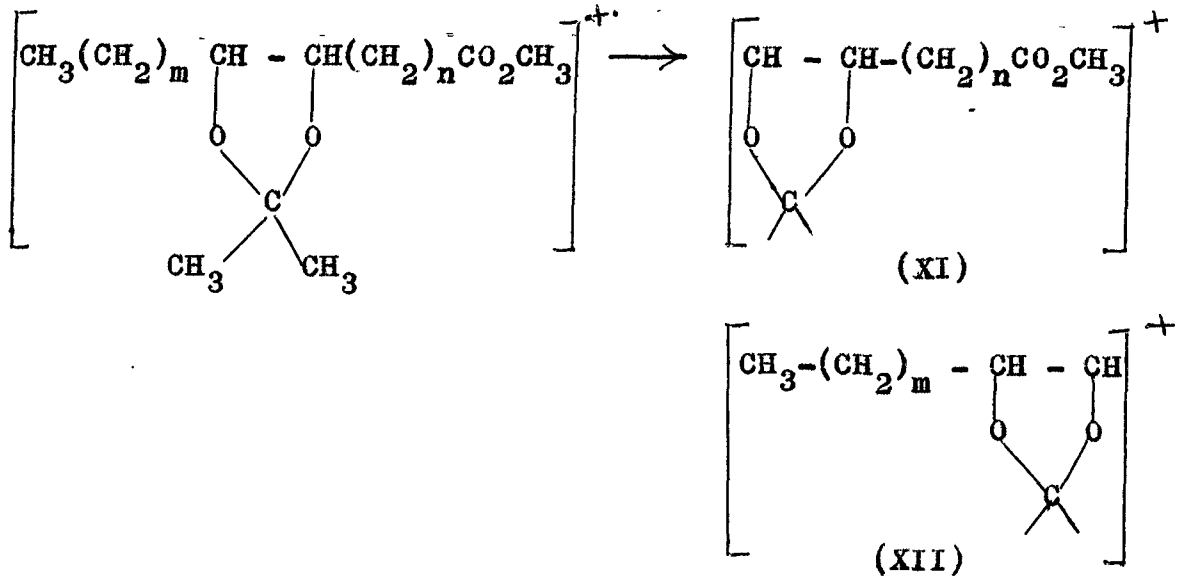
by mass spectrometry of the corresponding dimethylamino alcohol derivative. Ryhage and Stenhagen[31] have observed that the mass spectrum of vicinal dihydroxy ester obtained by the hydrolysis of epoxide of the original unsaturated compound reveals a prominent cleavage of the carbon-carbon bond between the two hydroxyl group. Thus the spectrum of methyl 9,10-dihydroxystearate has an intense peak at m/e 187 corresponding to ion (X). This fragment tends to lose methanol to form another prominent peak at m/e 155. The hydrogen atom which is expelled together with the methoxy group is, to a large extent, the labile hydrogen from the hydroxy group.



(X)

McCloskey and McClelland[32] studied the mass spectra of 1,3-dioxolane derivative of a vicinal dihydroxy ester. The mass spectrum of this derivative has two peaks (ions (XI) and (XII)) which correspond to the cleavage of the bond at either side of the ring. They suggested that the spectra of dioxolane derivative can be used to distinguish between two geometrical isomers. In the spectrum of erythro isomer which is derived from a cis-unsaturated ester, the peak M-89 is at least twice as intense as that in the spectrum of the threo isomer derived from trans-

unsaturated ester.



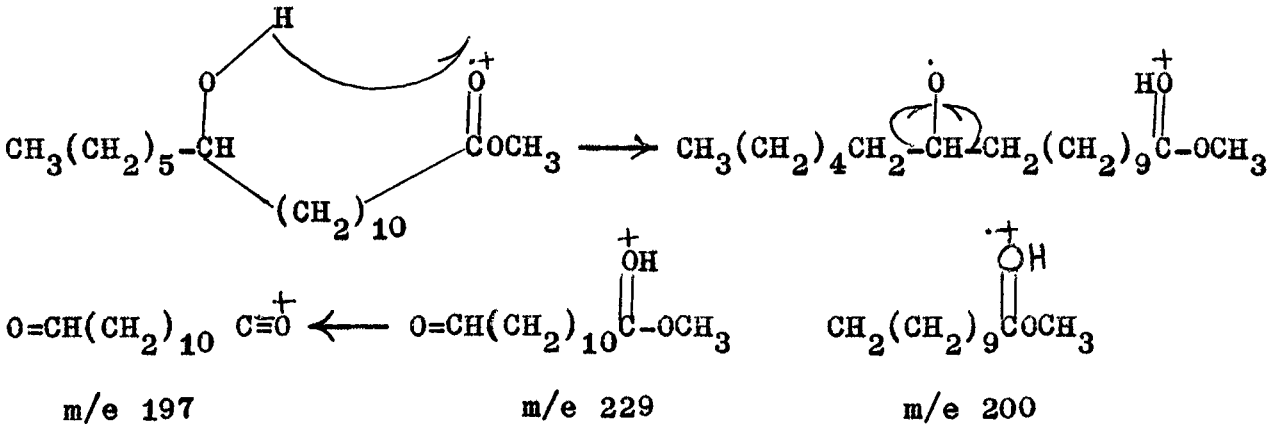
(XI)

(XII)

More recently Niehans and Ryhage[33] developed a technique for the determination of double bond positions in polyunsaturated fatty acids by combination gas chromatography-mass spectrometry. The polyhydroxy acids were converted to polymethoxy methyl esters with dimethyl sulfinyl carbanion and methyl Iodide. The mass spectra of 9,10-dimethoxy ethyl hexadecanoate derived from palmitoleic acid showed no molecular ion peak. But significant peaks at m/e 229 $[M-(31+32)]$, and m/e 235 $[M-(31 + 2 \times 32)]$, were observed. These peaks probably resulted by the loss of ($-OCH_3$) and successive loss of elements of methanol. The determination of the positions of the methoxy group help in the location of the double bond in the original fatty acid.

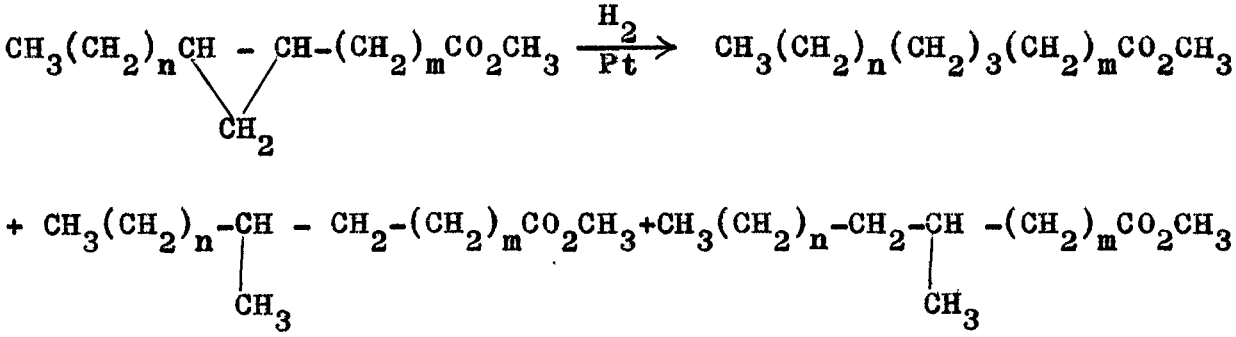
Some Applications of Mass Spectrometry.

In recent years few papers have appeared describing the use of mass spectrometry in the detection and characterization of unusual fatty acids and their derivatives. Wolff and coworkers[34] reported the results of the mass spectrometry of long chain hydroxy esters and their deuterium labelling analogues, using methyl 12-hydroxyoctadecanoate as a model compound. Three prominent peaks were observed in the mass spectra of this compound. Of primary interest are the ions due to α-cleavage m/e 229 and the product of subsequent elimination of methanol m/e 197. The ion m/e 200 corresponds to cleavage of the 11,12 bond to the hydroxy function, accompanied by hydroxy hydrogen transfer to the carbomethoxy charge bearing fragment. The genesis of the ions as suggested by the authors follows the mechanism as given under:



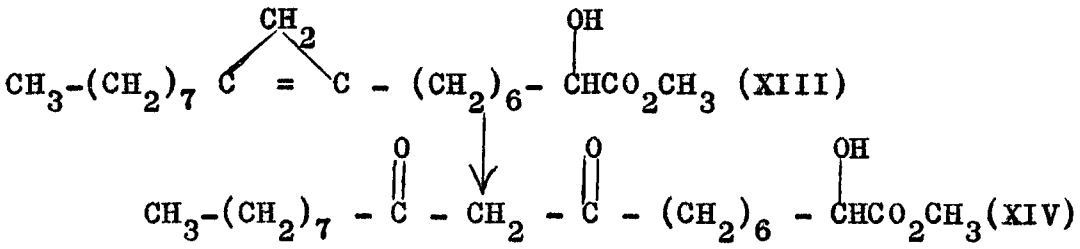
McCloskey and Law[35] recently have used the mass spectro-

metry for the ring location of the cyclopropane ring in fatty acid ester. The technique involves reductive ring opening to give a mixture of branched-chain and straight chain acid esters which were subsequently analysed with a combination of gas chromatography - mass spectrometry. The presence of a methyl branch can be recognized by the appearance of a peak of low relative intensity at M-15 probably due to loss of a methyl branch. Thus the examination of the spectra obtained from the mixture of branched-chain acid esters permit assignment of the position of methyl groups and hence the position of the ring in the parent compound.



Morris and Hall[36] have characterised an unusual acid D-2-Hydroxysterculic acid (XIII) in Pachira and Bombacopsis seed oils with the help of the mass spectra. The parent peak in the mass spectrum indicated a molecular weight of 356, which also corresponds to structure (XIV). The position of the β-diketo grouping in the chain could be deduced from the mass spectrum. Peaks at m/e 198 and m/e 258, due to rearrangement ions from β-cleavage on one or the other side of the β-diketo

grouping, showed that the keto groups were in the 9- and 11-positions of the chain, as illustrated in the following equation.



More recently Christie and coworkers[37] observed that mass spectrometry could be used in the identification of cyclopentenyl fatty acids and their derivatives. They observed that the mass spectra of cyclopentenyl fatty esters are distinct from those of corresponding normal unsaturated and saturated esters. The principal mode of cleavage is β to the 5-membered ring, whereas with the corresponding saturated compounds, cleavage is adjacent to the ring. Prominent peaks of the spectrum of methyl hydnocarpate are the molecular ion peak (M=266), m/e 185 (cleavage β to the cyclopentene ring), m/e 153 ($CH_2 = CH\ (CH_2)_7CO-$), and m/e 82.

Eliasson and coworkers[38] have carried out mass spectrometric investigations during the course of their work on the synthesis and the resolution of 2-hydroxyundec-10-enoic acid. The mass spectrum of its methyl ester was found to be identical with that of the antipode. The spectrum showed a molecular ion peak at m/e 214, expected for a monounsaturated hydroxy substi-

tuted C11 methyl ester.

In the field of fatty acids no extensive work has been done on the chemistry of unusual acids. The recent trend is to study the reaction conditions, product isolation and mechanism involved in the simple model compounds. It is with this view that some reactions of a terminal olefinic acid have been chosen for the subject of the present investigation.

(A). Studies on Solvomercuration and Demercuration of 10-Undecenoic Acid.

Since the discovery of the oxymercuration of olefins by Hofmann and Sand[39] in 1900, the addition of mercuric salt or of the elements of mixed mercuric salt Hg(OR)X, to an olefinic double bond has been developed into a very useful reaction for the separation of unsaturated fatty acids and for the synthesis of certain organic compounds.

$$-CH=CH- \xrightarrow[CH_3OH]{Hg(OAc)_2} -\underset{OCH_3}{CH}-\underset{HgOCOCH_3}{CH}-$$

The above reaction gives quantitative yield of the adducts from which the starting material can be recovered by the decomposition of the adducts with a mineral acid. Mangold and Kammereck[40] converted the unsaturated acids into mercuric acetate adducts which were then separated from the unreacted saturated components and from each other depending upon the number of double bonds present in the original acid. The mercuric adduct method has also been used to separate cis, trans pairs of fatty acids. The success of the separation is based on the fact that mercuric acetate reacts twenty times faster with cis- than with trans-olefinic bonds.

Although an enormous number of studies of the oxymercuration reaction have appeared in the literature the later papers have concerned themselves primarily with the mechanism of the reaction and stereochemistry of the products. Stoichiometrically the oxymercuration reaction consists in the addition of mercuric salt to an olefinic double bond. A reduction of carbon-mercury bond (demercuration) gives the corresponding alcohol, ether or ester.

$$-C = C- + Hg(OR)X \longrightarrow -\underset{OR}{\overset{|}{C}}-\underset{HgX}{\overset{|}{C}} \xrightarrow{(H)} -\underset{OR}{\overset{|}{C}}-\underset{H}{\overset{|}{C}}-$$

From the work of Hofmann and Sand[39], it is known that in the case of simple olefins, the reaction proceeds in the Morkovnikov sense (that is, by placing the mercury atom on the carbon having the more hydrogen) to give an almost quantitative yield of product. The oxymercuration and demercuration sequence since then has been used to prepare the Morkovnikov alcohol from a wide variety of olefins. In the synthetic work it compares well with the hydroboration-oxidation of olefins. The latter reaction is now known to provide a highly convenient procedure without evident rearrangement, for achieving the anti-Morkovnikov hydration of carbon-carbon double bonds. Thus the oxymercuration and demercuration reaction has a wide scope applicable to a large number of structural types. The reaction displaces

high specificity and sensitivity to steric factors and advantage of these characteristics can be taken to achieve desired syntheses.

In recent years this combined procedure has been used with wide variations in experimental conditions with a view to use it as general technique for the introduction of wide variety of nucleophiles to carbon-carbon double bond in the Morkovnikov direction. For example a number of solvents has been used from time to time so much so that the initial reaction is now designated as solvomercuration. Reduction of the carbon-mercury bond has been achieved using a number of reagents, including sodium mercury amalgam, hydrazine, lithium aluminium hydride, and various borohydrides. Thus it has been reported that the solvomercuration-demercuration of representative olefins in the presence of water provides a convenient procedure for the Morkovnikov hydration of the carbon-carbon double bond to form alcohols[41]. Similarly the use of alcohols as a solvent provides a highly convenient route to the corresponding ethers[42]. Brown and Kurek[43] have reported that the solvomercuration-demercuration of olefins in the presence of acetonitrile provides a convenient technique for the Morkovnikov amination of carbon-carbon double bond. In this amidomercuration reaction mercuric nitrate was found to give highly satisfactory results.

$$R - CH = CH_2 + CH_3CN + Hg(NO_3)_2 \longrightarrow R - \underset{\underset{\underset{ONO_2}{|}}{N=CCH_3}}{\overset{}{|}}{CH}CH_2-HgNO_3$$

$$R - \underset{\underset{\underset{ONO_2}{|}}{N=C.CH_3}}{\overset{}{|}}{CH} - CH_2HgNO_3 \xrightarrow[1/4\ NaBH_4]{2NaOH} R - \underset{\underset{NHCOCH_3}{|}}{\overset{}{|}}{CH} - CH_3 + Hg + 2NaNO_3 + 1/2\ NaB(OH)_4$$

In these earlier work the initial oxymercuration product was separated and this intermediate is reduced in a separate operation. Very recently Brown and coworker[44] reported that the oxymercuration can be carried out quantitatively and rapidly in aqueous tetrahydrofuran and that the mercury could be removed in situ in second fast reaction by treatment with alkaline sodium borohydride. This reaction is a convenient Morkovnikov hydration procedure based on hydroboration-oxidation.

$$R - CH = CH_2 + Hg(OAC)_2 \xrightarrow[25^{\circ} \sim 10\ min.]{50:50,\ THF-H_2O} R - \underset{\underset{OH}{|}}{\overset{}{|}}{CH} - CH_2-HgoAC$$

$$R - \underset{\underset{OH}{|}}{\overset{}{|}}{CH} - CH_2HgoAC \xrightarrow[25^{\circ}]{NaOH,\ NaBH_4} R - \underset{\underset{OH}{|}}{\overset{}{|}}{CH} - CH_3 + Hg$$

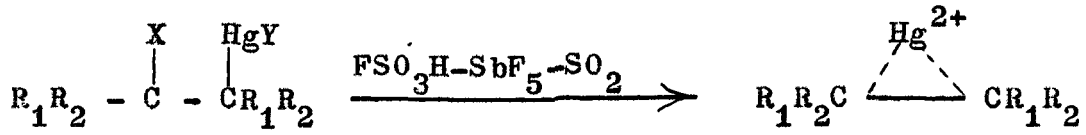
Under the conditions of hydration (much shorter reaction time) the undesired side reactions are not observed. These authors have developed the procedures for the synthesis of ethers[42] and

amines[43] and others have utilized the technique for the synthesis of alkyl azides[45] and peroxides[46].

Although numerous kinetic and stereochemical investigations have been conducted for a detailed study of the solvomercuration reaction, the nature of reaction intermediate and/transition state involved in the addition reactions in many systems is still unresolved. Frederick et al[47]. in their study of the reduction of alkyl mercury hydroxide by sodium borohydride proposed a four centered transition state. Their observation of retention of configuration and incorporation of deuterium rather than hydrogen (using $NaBD_4$) in their experiment provided additional evidence against SN^2 type and carbanion mechanisms.

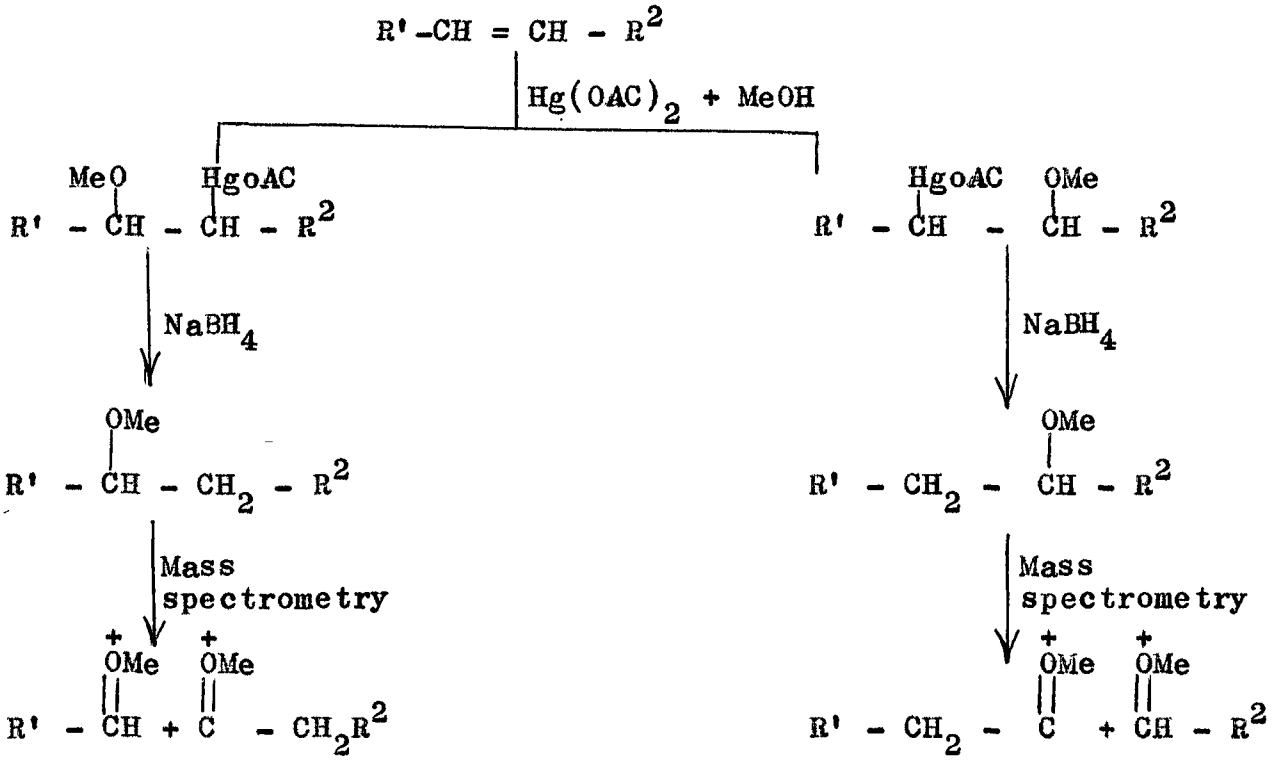
In 1939 Lucas et al.[48] proposed that the oxymercuration of alkenes involves a trans addition(by analogy with the bromination of alkenes), and that the trans addition proceeded via a cyclic cationic intermediate, or mercurinium ion, which underwent ring opening by nucleophilic attack at carbon with inversion. In a recent kinetic study of this reaction Halpern and Tinkler[49] could not obtain enough evidence for or against the intermediacy of mercurinium ions as reaction intermediate. Since the introduction of mercurinium ion concept by Winstein[48] several other descriptions of this species and other supposedly closely related species, have appeared in the literature. It was only recently

that Olah and Clifford[50] reported the first direct observation of mercurinium ion formation that exists as stable long lived intermediate. The ions were produced by treating organomercurials of the type I with FSO_3H-SbF_5-SO_2 solution.



I

Methoxymercuration demercuration has long been used as a means of separating long chain esters differing in unsaturation[51] and, more recently, as a method of "labelling" double bonds prior to mass spectrometry examination. According to Abley and coworkers[52] the position of double bonds in mono and polyenoid long chain esters may be conveniently determined by reduction of their methoxymercuration product with sodium borohydride followed by combined gas chromatography-mass spectrometry of the resulting methoxylated esters. The above procedure have been applied to methyl oleate, elaidate, linoleate and linolenate as under:



The position of the double bonds in methyl oleate and elaidate is revealed by the intense ions in the mass spectra of their methoxylated derivatives at m/e 157, 171, 201, and 215 which represents the fragments $CH_3(CH_2)_n\overset{+}{C}HOMe$ (n=7,8) and $Me\overset{+}{O}$ - $CH(CH_2)_mCO_2Me$ (m=7,8) respectively.

Recently Gunstone and Inglis[53] carried out oxymercuration and demercuration studies on methyl oleate, methyl linoleate and hendec-10-enoate. The latter gave only one product: methyl 10-methoxy hendecanoate (86%) while others gave 1:1 mixture of methoxy stearates. They have further observed that the presence of a substituent (OH, OMe, OAc) affects the proportion of two isomeric products formed during the reaction. In a recent short

communication[54] they have also deviced a new procedure for the identification, analysis and isolation of long chain alcohols and acids having alkene unsaturation at position 3, 4 or 5.

Present Work.

The present work deals with the solvomercuration studies on a terminal olefinic fatty acid, 10-undecenoic acid, with a view to prepare the corresponding alcohol, ethers, amine and hydroxy ethers. Water, methyl and ethyl alcohols, acetonitrile and ethylene and propylene glycols have been used as nucleophilic solvents. It was observed that the solvomercuration-demercuration deals with the addition to the double bond in the Markovnikov direction without isomerization or rearrangement. Samples of extra pure 10-undecenoic acid (E. Merck) and mercuric acetate (BDH) were used in the present study. All solvents used were predried. The discussion of the results obtained by the use of various solvents are given below under separate headings.

(a) Markovnikov hydration of 10-undecenoic acid (XV).

This reaction deals with the oxymercuration of undecenoic acid with mercuric acetate in presence of a 50:50 tetrahydrofuran-water medium, followed by in situ reduction of the oxy-

mercurial with sodium borohydride in presence of sodium hydroxide. The procedure of solvomercuration-demercuration adopted in the present investigation is essentially that of Brown and Geoghegan[44] The reaction of undecenoic acid (XV) with mercuric acetate using equimolecular proportions and the alkaline $NaBH_4$ reduction of the oxymercurial finally yielded a crystalline solid (XVI), m.p. 49°. The fact that this hydration product is a single isomer was shown by a single spot on a thin layer chromatographic (TLC) plate. It was characterized as 10-hydroxyundecanoic acid by its i.r. and n.m.r. data and its conversion to 10-ketoundecanoic acid (XVII). Its i.r. spectrum showed the presence of a hydroxyl moiety (3440 cm^{-1}) and a carboxyl function (1710 cm^{-1}). Its n.m.r. spectrum gave five signals integrated for 22 protons. The spectrum indicated a saturated penultimate hydroxyl structure because the terminal C-methyl protons were observed slightly downfield at 8.83 τ due to the deshielding effect of the hydroxyl group. The hydroxyl and carboxyl protons that appeared at 2.9 τ (singlet) are known to give a combined peak in hydroxy acids[55]. Disappearance of the sharp singlet at 2.9 τ after deuterium exchange is evidence that the hydroxyl and carboxl protons signals are incorporated in this peak. The methine proton appeared as a multiplet at 6.24 τ .

Jones[56] oxidation of the 10-hydroxyundecanoic acid (XVI) readily yielded the corresponding keto acid (XVII), 10-keto-

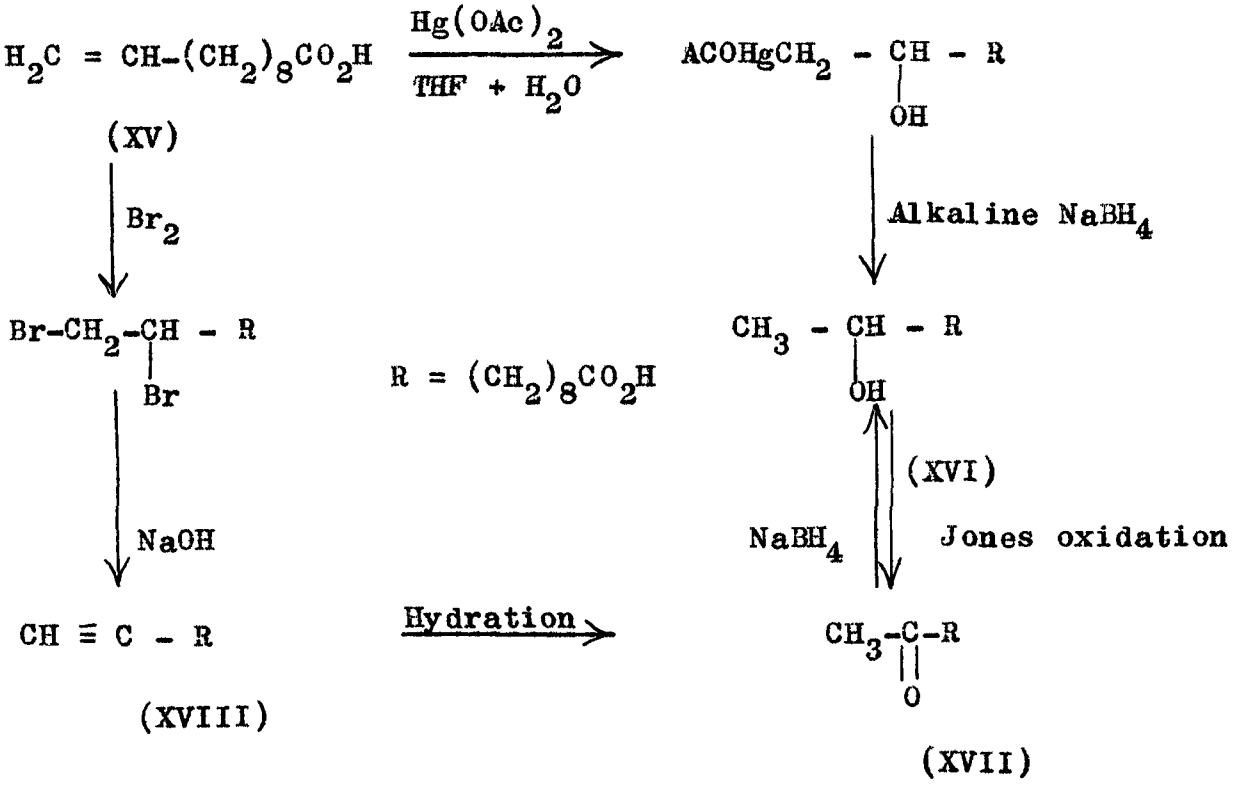
undecanoic acid, m.p. 56-57°. Its 2,4-dinitrophenyl hydrazone derivative had m.p. 115-116° and its semicarbazone melted at 136°. The structure assigned to this Jones oxidation product was further supported by its i.r. and n.m.r. spectra. IR spectrum showed carbonyl absorption band at 1720 and carboxyl band at 1710 cm^{-1}. The n.m.r. spectrum of this keto acid showed three peaks of significance. The signal for four protons due to the α-methylene to carbonyl and methylene flanking the carboxyl group appeared merged at 7.64 τ as an unsymmetrical quartet. A sharp peak due to the terminal methyl appeared at 8.7 τ instead of the usual 9.1 τ because of the deshielding effect of 10-keto group. A broad one proton singlet ascribable to carboxyl proton appeared at 1.1 τ , which was found to be extinguished by the addition of deuterium oxide (D_2O).

A partial synthesis of Markovnikov hydration product, 10-hydroxyundecanoic acid (XVI) was achieved starting from the parent acid undecenoic acid (XV). The latter acid on bromination and dehydrobromination by the usual way yielded the terminal acetylenic acid (XVIII), 10-undecynoic acid m.p. 41-42°. The acetylenic structure was established by its characteristic i.r. band at 2122 cm^{-1}. Further among its n.m.r. signals, the terminal acetylenic proton appeared as a sharp singlet at 7.7 τ and the four methylene protons adjacent to the acetylenic as well as carboxyl functions appeared as a well resolved triplet at 7.8 τ .

The 10-undecenoic acid (XV) on hydration[57] by dilute sulphuric acid yielded 10-ketoundecanoic acid (XVII) m.p. 56-57°. A mixed m.p. with the keto acid prepared by the Jones oxidation of 10-hydroxyundecanoic acid (XVI) showed no depression. The i.r. as well as n.m.r. spectra of the two keto acids were found to be identical. The $NaBH_4$ reduction of 10-ketoundecanoic acid (XVII) gave a hydroxy acid (XVI) which melted at 49° and was found to be identical in all respects with the Markovnikov hydration product, 10-hydroxyundecanoic acid.

The above reactions are summarized in Scheme-1.

Scheme - 1.



(b) Morkovnikov amination of 10-undecenoic acid (XV).

In an attempt to bring about amination of the double bond the solvomercuration-demercuration of 10-undecenoic acid in the presence of acetonitrile was carried out following the procedure of Brown and Kurek[43]. Undecenoic acid was reacted with mercuric nitrate in presence of acetonitrile and the resultant adduct was subjected to alkaline $NaBH_4$ reduction as usual. The sodium salt of the corresponding N-alkylamide on treatment with sulphuric acid yielded an oily product. On purification over silica gel G column it crystallized to yield pure 10-aminoundecanoic acid, m.p. 38-40°. This amination product was TLC homogenous. Its structure was established from the study of its i.r. and n.m.r. spectra and its conversion to 10-hydroxyundecanoic acid. Its i.r. spectrum showed the characteristic absorptions for the amino group at 3415 and 1415 cm^{-1}. Its n.m.r. spectrum showed six signals in all, integrated for 23 protons. Two significant signals, one at 3.75 τ (singlet for two protons) of $-NH_2$ protons and the other at 6.25 τ (multiplet for one proton) of C10 methine proton were observed. The signal for the acid proton was observed at 0.9 τ . Disappearance of the signals at 3.75 τ and 0.9 τ after deuterium exchange showed that the amino protons signal is incorporated at 3.75 τ . The 10-amino structure of the amination product was further supported by its conversion to 10-hydroxyundecanoic acid by treatment with

dilute hydrochloric acid. This hydroxy acid was found to be identical with that obtained by oxymercuration-demercuration of original 10-undecenoic acid as described earlier. The ready formation of the amino substituted acid supported the earlier observation[43] that Markovnikov amination could be brought about by the Solvomercuration-demercuration of olefins in the presence of acetonitrile.

(c) Solvomercuration-demercuration using alcohols as nucleophilic solvents.

In the literature reports are available of the use of monohydric alcohols as nucleophilic solvents in the solvomercuration-demercuration of the representative olefins. It is known that the above procedure is a convenient synthetic route for the conversion of olefins into Markovnikov ethers. The present work deals with the use of monohydric as well as dihydric alcohols in the solvomercuration-demercuration of a terminal olefinic acid.

10-Undecenoic acid (XV) was treated with mercuric acetate in presence of methanol, ethanol and isopropanol respectively according to the procedure of Brown and Rei[42]. The alkaline $NaBH_4$ reduction of the mercurial intermediates followed by the purification of the corresponding ethers by column chromatography finally yielded 10-methoxy and 10-ethoxyundecanoic acid (yield~90%)

as colorless oils. In each case a single spot on TLC was observed, which showed that only one isomeric ether was obtained. The assignment of 10-methoxy and 10-ethoxy structure was supported by spectroscopic data. The i.r. spectra of both the products gave the characteristic ether absorption at 1110 cm^{-1}. Besides the usual n.m.r. signals as described earlier a signal at 6.8 τ (for 3 protons) was observed in the product obtained by the use of methanol. This showed the presence of methoxy group. In the case of the product obtained by the use of ethanol two significant signals were observed. A signal at 6.7 τ (multiplet integrated for 3 protons) appeared for the $-OCH_2$ and C10 methine protons. Another six proton multiplet appeared at 8.93 τ which suggests that the terminal methyl and ethoxy methyl signals have merged together. The presence of methoxy and ethoxy functions at the C10 of the fatty acid chain was thus established. Therefore it follows that the procedure of solvomercuration-demercuration can satisfactorily bring about the conversion of olefins into ethers in the Markovnikov direction.

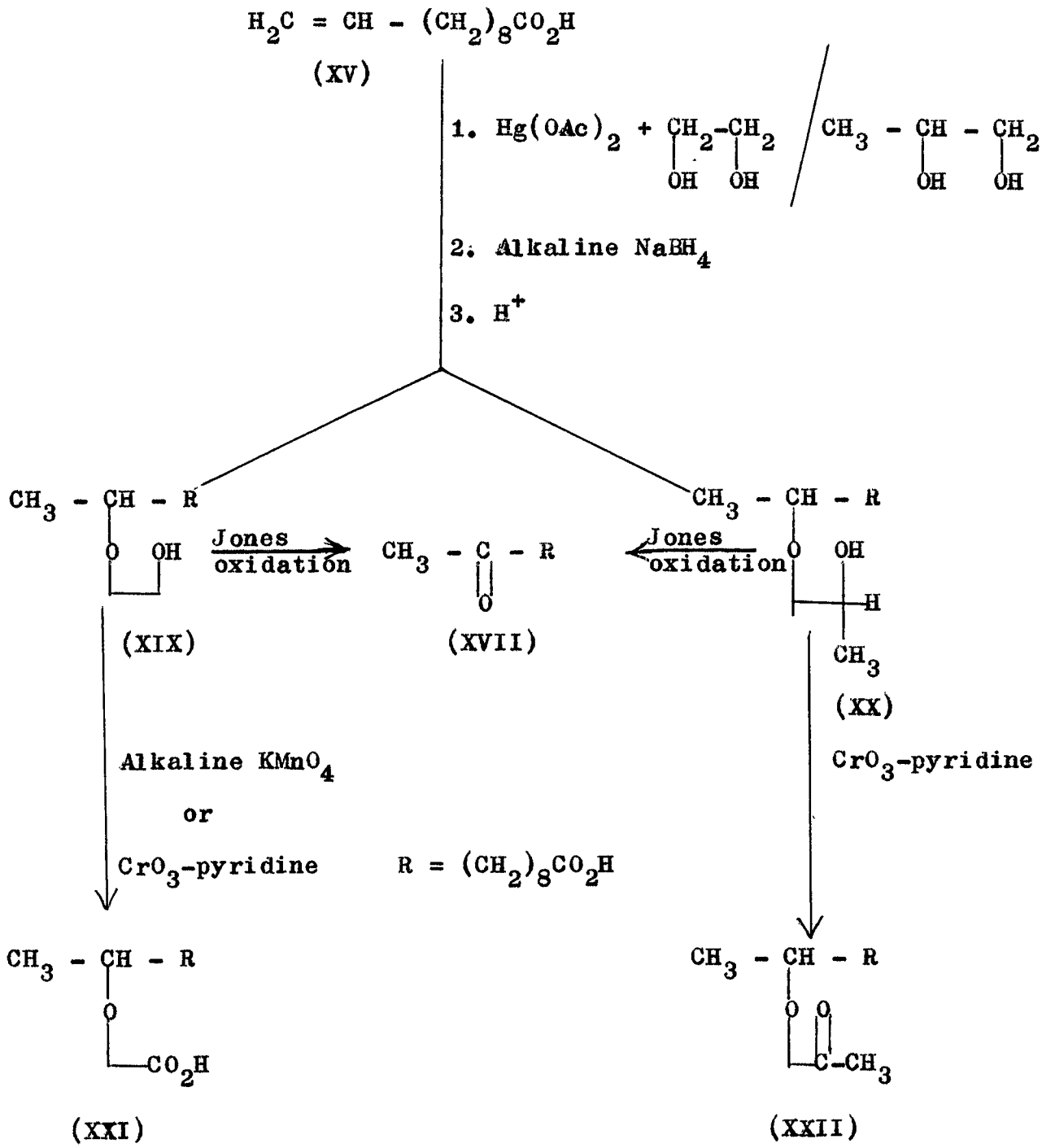
The above reaction of 10-undecenoic acid under the same conditions did not proceed in the presence of isopropanol as indicated by TLC. The non-formation of the corresponding ether is presumably due to the bulky isopropyl moiety which hinders the formation of mercurial adduct.

The use of dihydric alcohols as nucleophilic solvents in

the solvomercuration-demercuration reaction has not been mentioned in the literature. In the present work it was found that in the presence of ethylene and propylene glycols the reaction proceeded as smoothly as in the case of primary alcohols. The yields however, were comparitively less.

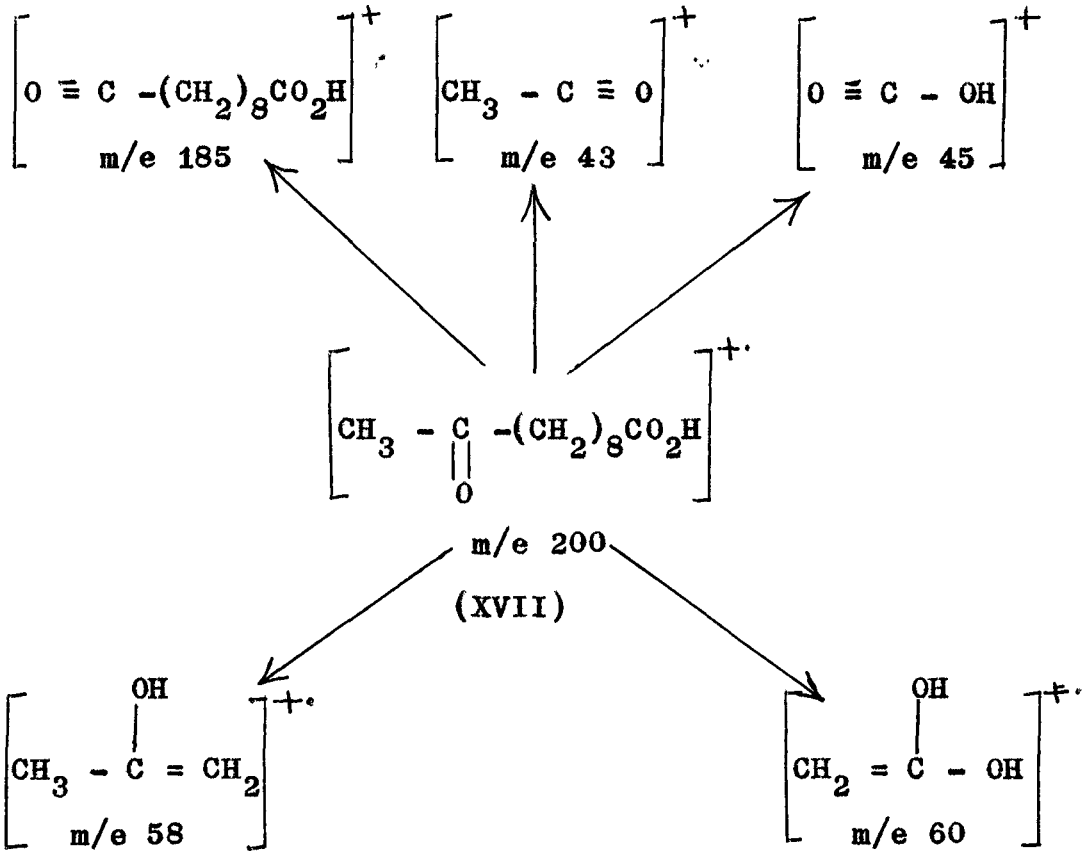
10-Undecenoic acid (XV) on solvomercuration-demercuration using ethylene glycol as nucleophilic solvent and subsequent in situ reduction by alkaline $NaBH_4$ readily yielded ($\sim$ 80%) 10-(2'-hydroxyethoxy)undecanoic acid (XIX). Similarly 10-(2'-methyl-2-hydroxyethoxy)undecanoic acid (XX) ($\sim$ 70%) was obtained by the use of propylene glycol. Identity of the hydroxy ethers (XIX) and (XX) was based on spectral data. The i.r. spectrum of both the hydroxy ethers showed peaks at 3410 (OH), 1110, 1056 cm^{-1} (C-O-). The n.m.r. of hydroxy ether (XIX) gave five signals integrated for 26 protons. The significant signal at 6.5 τ (multiplet, 5 protons) is ascribable to C10-methine and C10-OCH_2CH_2-protons. The n.m.r. spectrum of hydroxy ether (XX) exhibited a multiplet centred at 6.6 τ (4 protons, C10-OCH_2-CHOH + C10 methine) and a signal at 8.84 τ (6 protons) ascribable to C11 methyl and 3'-methyl protons. The hydroxy ethers obtained from 10-undecenoic acid by the use of ethylene and propylene glycols are shown in Scheme-2.

Scheme - 2.


H2C = CH - (CH2)8CO2H
(XV)
1. Hg(OAc)2 + CH2-CH2 (OH OH) / CH3 - CH - CH2 (OH OH)
2. Alkaline NaBH4
3. H+
CH3 - CH - R (O OH)
Jones oxidation
CH3 - C - R (O)
Jones oxidation
CH3 - CH - R (O OH H, CH3)
(XIX)
(XVII)
(XX)
Alkaline KMnO4
or
CrO3-pyridine
R = (CH2)8CO2H
CrO3-pyridine
CH3 - CH - R (O CO2H)
CH3 - CH - R (O O C-CH3)
(XXI)
(XXII)

An attempt to obtain the carboxy ether (XXI) by mild oxidation of the hydroxy ether (XIX) led to very interesting results. Oxidation of the hydroxy ether (XIX) with Jones reagent gave the unexpected 10-ketoundecanoic acid (XVII) whereas oxidation by alkaline potassium permanganate/or chromium trioxide-pyridine yielded the desired carboxy ether (XXI). The identity of the Jones oxidation product (XVII) was established by a comparison of its m.p., i.r. and n.m.r. spectra with that of 10-ketoundecanoic acid prepared earlier via the Markovnikov hydration procedure. The structure of the keto acid (XVII) was further supported by mass spectrometry. Its mass spectrum gave a molecular ion peak at m/e 200 ($C_{11}H_{20}O_3$). The other significant peaks obtained were at m/e 185, 182, 60, 58, and 43. The peak at m/e 182 is attributed to the loss of water from the molecular ion. The keto acid (XVII) is split on both sides alpha to C10 carbon resulting ions m/e 185 and 43. The preferential formation of the base peak ion m/e 43 may be attributed to the loss of bulky group comprising the fatty acid chain. The peak at m/e 45 appeared due to fragment arising from α-cleavage with respect to carboxyl group, whereas the peak at m/e 58 and 60 are the result of β-cleavage with respect to C10-carbonyl and carboxyl groups respectively as shown in Scheme-3.

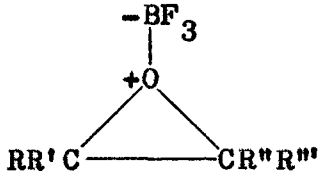
Scheme - 3.



The hydroxy ether (XX) similarly on oxidation with CrO_3-pyridine yielded the desired ketonic product (XXII). Jones[56] oxidation of hydroxy ether (XX) under the same conditions gave again the unexpected 10-ketoundecanoic acid (XVII). Thus in both the hydroxy ethers (XIX) and (XX) Jones oxidation gave the same keto acid (XVII), the formation of which presumably is due to acidic medium used. This reaction course appears to be very unusual as far as the role of Jones reagent in the oxidation of hydroxy ether is concerned.

(B) Borontrifluoride Catalysed Reactions of Epoxides.

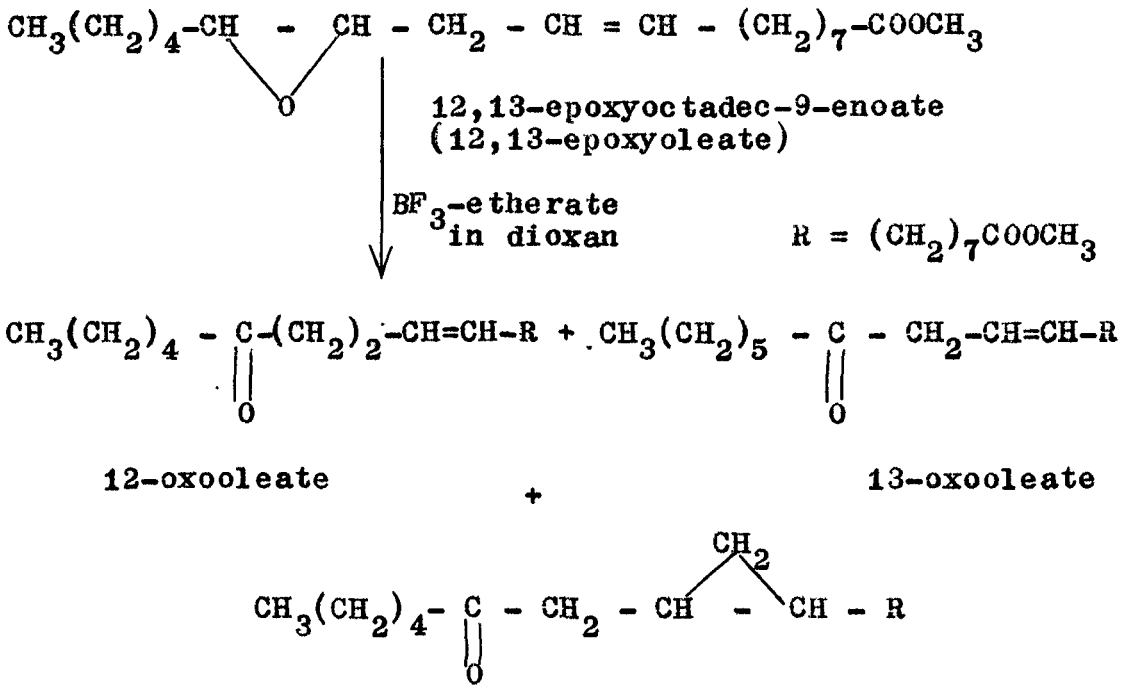
The ring opening reactions of epoxides generally involve attack on an epoxide carbon atom by an external nucleophilic reagent. If such a reagent is not available, its place may be taken by a substituent group in the epoxide molecule. The group migrates from one epoxide carbon atom to other, with production of an aldehyde or ketone. Rearrangement reactions of this kind are usually catalysed by Lewis acids (e.g., $MgBr_2$/ether, BF_3/ether) but may also be brought about by the action of heat alone. Since the reactions are normally catalysed by a Lewis acid, the entity which rearranges is not the epoxide itself, but its complex with the acid, e.g.,



Rearrangement reactions can be considered as a special case of ring-opening reactions where, in the absence of an external nucleophile, one of the epoxide carbon atoms is attacked by a neighbouring group or atom in the same molecule.

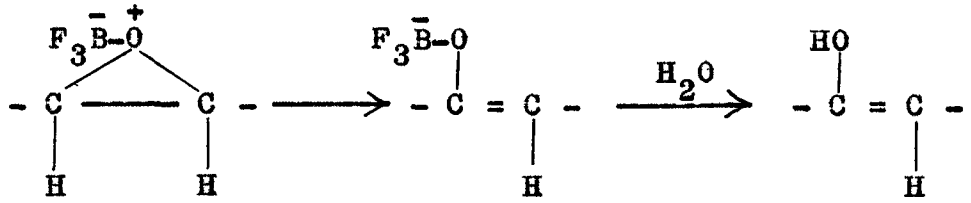
The use of borontrifluoride in the rearrangement of fatty epoxy esters to keto esters was first made by Wallens and coworkers[58]. They determined the optimum conditions for rearrangement of epoxides to ketones by using methyl 9,10-epoxystearate

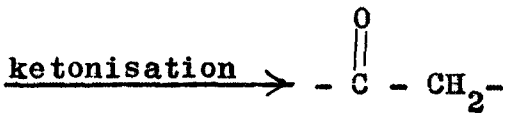
and then applied to other epoxy compounds. In a subsequent work on the action of BF_3-etherate on unsaturated epoxy compounds Canonica et al.[59] reported that the reaction of methyl 12,13-epoxyoctadec-9-enoate and methyl 9,10-epoxyoctadec-12-enoate with BF_3-etherate in benzene yielded cyclic and unsaturated acyclic compounds. Their conclusions were confirmed in a simultaneous publication by Conacher and Gunstone[60]. They observed that methyl 12,13-epoxyoctadec-9-enoate when treated with BF_3-etherate in dioxan solution yielded 12- and 13-oxo-oleates as major products. These are accompanied by cyclopropane compounds which were identified as the cis- and trans-isomers of methyl 9,10-methylene-12-oxoheptadecanoate. In benzene solution the cyclopropane derivatives are formed in higher yields. These reactions are summarized below:



Cis and trans, methyl 9,10-methylene-12-oxo-heptadecanoate.

They postulated that the reaction probably occurs through the enolic form of the ketone which would arise through the changes shown below:





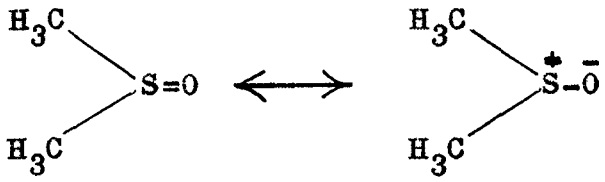
Thus it is established that the treatment of fatty epoxide with Lewis acid usually leads to the complete disappearance of epoxide function and to the formation of ketones as major products. The extent to which the desired reaction, isomerization to ketone, is attained depend largely upon the catalyst, the reaction conditions and the amount of water present.

More recently Kolaczinski and coworkers[61] have studied the isomerization of unbranched 1,2-epoxides with Lewis acid. The conversion of long chain unbranched 1,2-epoxide, exemplified by 1-n-octene epoxide, to the corresponding aldehyde in good yield was obtained at conditions of high dilutions, short reaction time and proper choice of catalyst. BF_3-etherate in dioxan was found to give the best results. From this work it is evident that a long chain terminal epoxide on BF_3 catalysed rearrangement leads

to the formation of an aldehyde, in which the carbonyl function is attached to the terminal carbon atom.

During the last few years BF_3 has been used as a catalyst for many reactions involving condensations, rearrangements and polymerizations. Because of its great tendency to form complex with oxygen compounds, BF_3 catalyses most successfully the reactions of oxiranes. Recently it has been used as a catalyst in the dimethyl sulfoxide (DMSO) oxidation[62] of epoxy compounds.

In a recent article Swern[63] discussed the nature of 1,2-dipolar addition of DMSO to the oxirane group on the basis of his results obtained in a mechanistic study of the reaction. DMSO is a highly polar molecule that exists as a resonance hybrid of the two limiting forms A & B.



(A) (B)

The dipolar form (B) indicates a high level of nucleophilicity for the oxygen atom. This was first demonstrated by Kornblum et al.[64] in 1957 when they showed that tosylates and phenyl halides are readily converted to carbonyls by reaction with DMSO and the base sodium bicarbonate.

$$ArCO\ CH_2X + DMSO \xrightarrow{NaHCO_3} ArCOCHO + (CH_3)_2S + NaX$$

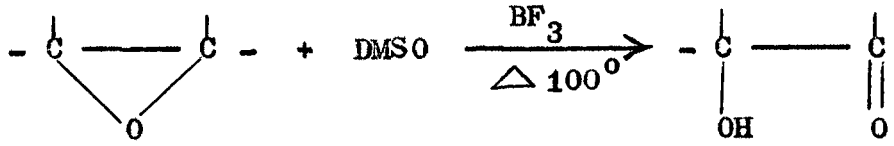
(X, halide or tosylate)

(a) $ArCOCH_2X + DMSO \longrightarrow ArCOCH_2O\overset{+}{S}(CH_3)_2 \; X^-$

(b) $ArCOCH_2O\overset{+}{S}(CH_3)_2 \; X^- \xrightarrow{NaHCO_3} ArCOCHO + (CH_3)_2S + NaX$

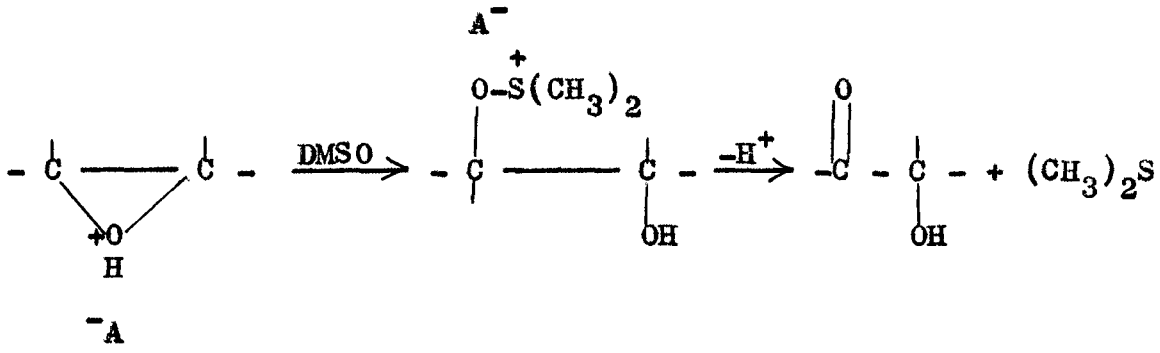
In the first step (a) of the above reaction DMSO performs a nucleophilic displacement on the substrate resulting in the formation of alkoxysulfonium salt as an intermediate. In the second step (b) the intermediate is finally converted to the carbonyl product and dimethyl sulfide.

The first report on the oxidation of epoxides by DMSO was made by Cohen and Tsuji[65] to obtain good yields of α-ketol from an epoxide using borontrifluoride as catalyst.



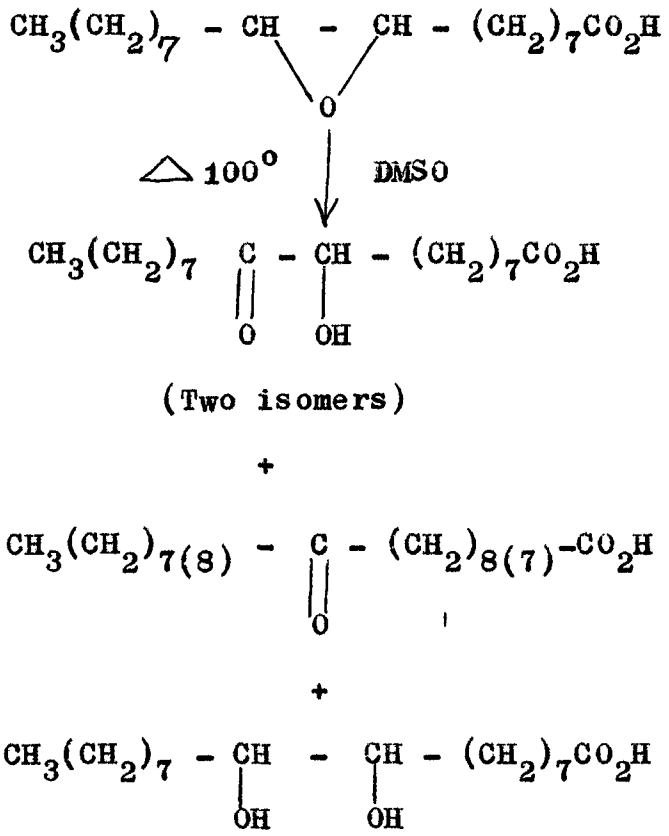
Later Tsuji[66] reported that the oxidation can also be effected without borontrifluoride if air is passed through the reaction mixture of epoxide and DMSO or if a catalytic amount of t-butyl hydroperoxide is present. He concluded that borontrifluoride catalysed reaction is ionic but that free radicals are involved in presence of air or hydroperoxides. To ascertain the nature of the reaction Swern[63] observed that the air or t-butyl hydroperoxide catalysed oxidation of styrene oxide by DMSO produces a small amount of a strong acid. When these reactions are attempted

in presence of excess sodium bicarbonate, oxidation does not take place. He concluded that in all cases previously reported the oxidation by DMSO is an acid catalysed ionic process and suggested the following mechanism:



The alkoxydimethyl sulfonium salt is the requisite intermediate which reacts with base to give ∝-ketol but with methanol or water as the nucleophile ∝-glycol is the oxidation product. The formation of glycol from oxiranes occurs with stereochemical inversion; cis-epoxides give threo glycols while trans- epoxides yield erythro glycols. Thus oxiranes can be converted to ∝-glycols in quantitative yield within a few minutes without the use of water. These reactions are illustrated with cis- and trans-9,10-epoxy stearic acid as under:

Cis or trans



A review of the current literature indicates that DMSO oxidation has been used in the long chain fatty compounds. Mahadevan[67] described the use of DMSO in the oxidation of tosylates of oleyl and elaidyl alcohols to their corresponding aldehydes. Later Mahadevan and coworkers[68] reported the synthesis of long chain saturated and unsaturated fatty aldehydes by the selective oxidation of sulfonate esters of the corresponding alcohols with DMSO in the presence of sodium bicarbonate. Eliane Brousse et al.[69] prepared a mixture of α-ketols 9(10)-hydroxy, 10(9)-keto octadecanoate by the oxidation of methyl cis-9,10-epoxy octadecanoate

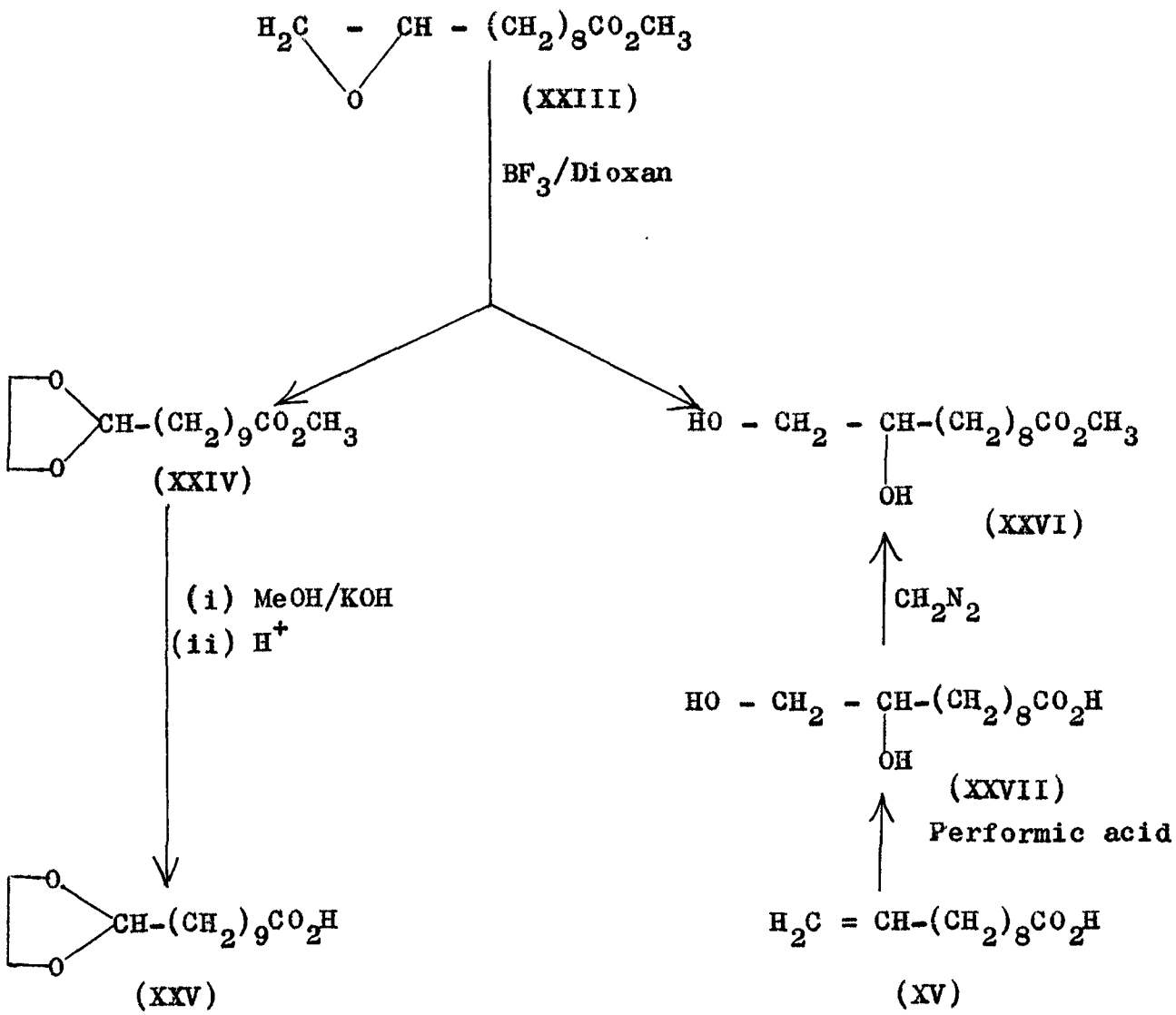
by DMSO in presence of catalytic amount of borontrifluoride-etherate.

Present Work.

From the foregoing account of rearrangement reactions of epoxides catalysed by BF_3, it is evident that no work has yet been done on the terminal epoxy derivatives of long chain fatty acids. An attempt to bring about rearrangement of methyl 10,11-epoxyundecanoate by the use of BF_3-etherate in dioxan was made in the present study following the procedure of Wallens and coworkers[58]. Thus methyl 10,11-epoxyundecanoate (XXIII) was first prepared by the perbenzoic acid epoxidation of methyl 10-undecenoate. The methyl 10-undecenoate prepared by the esterification of the acid was treated with perbenzoic acid in chloroform. After the usual work up the epoxy ester was obtained as a colorless oil responding to epoxy test by picric acid TLC[70]. It showed a three proton multiplet centred between 7.4 - 7.6 τ which was indicative of the protons attached to the oxirane oxygen. The i.r. spectrum clearly showed the epoxy absorption at 830 and ester carbonyl at 1740 cm^{-1}. The attempted rearrangement of this epoxy ester using BF_3-etherate in dioxan did not yield the desired rearranged carbonyl derivative analogous to those reported earlier[58] in the case of nonterminal epoxy fatty esters. On the other hand, the crude mixture from the

reaction of methyl 10,11-epoxyundecanoate (XXIII) with BF_3-etherate in dioxan, when chromatographed over silica gel G column resulted in the separation of a viscous oil (yield ~ 75%, major product) and a solid (yield ~25%, minor product). The two products were identified as methyl 11,11-ethylenedioxyundecanoate (XXIV) and methyl 10,11-dihydroxyundecanoate (XXVI) respectively as outlined in Scheme 4.

Scheme - 4.



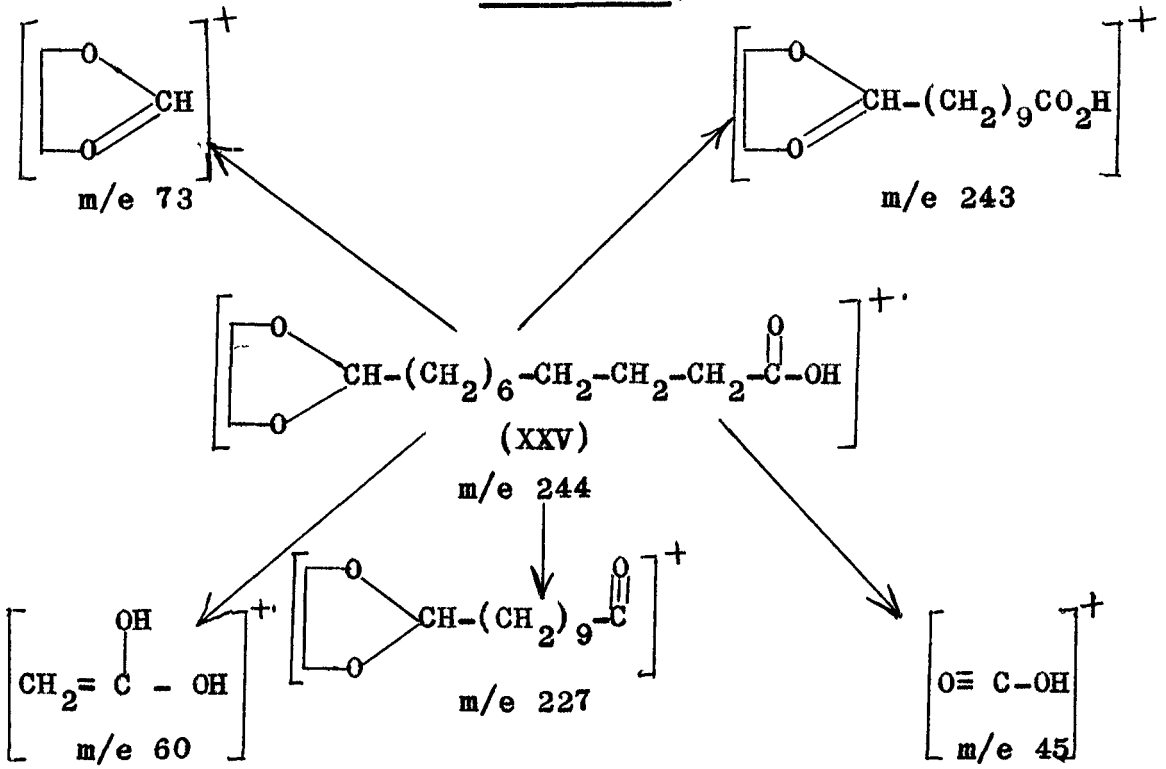
It is interesting to note that the liquid as well as the solid product were found to devoid of free carbonyl groups. The elemental analysis of (XXIV) corresponded to the molecular formula $C_{14}H_{26}O_4$. The identity of the product (XXIV) was based on spectral data. Its i.r. spectrum showed peaks at 1740 (ester carbonyl) and 1110, 1040, 1225, 1255 cm^{-1} (C-O-), the latter indicating possibly the presence of ether function.

Its n.m.r. spectrum is in favour of structure (XXIV). The four proton symmetrical multiplet between 6.1 - 6.25 τ is attributable to a dioxolane system ($-O-CH_2-CH_2-O-$) whereas a one proton triplet centred at 5.2 τ is indicative of the proton attached to the carbon (C11) bearing two oxygens. The methoxy protons appeared at 6.37 τ . The n.m.r. spectra of 1,3-dioxolanes reported in the literature[71] show that the signals arising from the $-O-CH_2-CH_2-O-$ proton of the ring appears at 6.03 τ and the signal of a proton attached to a carbon atom bearing two oxygen substituents appear at a lower field (5.08 τ). From a consideration of these spectral data and the molecular formula of the compound ($C_{14}H_{26}O_4$), the ester (XXIV) appeared to be a cyclic compound characterized as methyl 11,11-ethylenedioxyundecanoate. The ester (XXIV) on treatment with methanolic potassium hydroxide followed by acidification under very mild conditions regenerated the corresponding acid 11,11-ethylenedioxyundecanoic acid (XXV) which melted at 66-68°. Its i.r. and n.m.r. spectra were almost

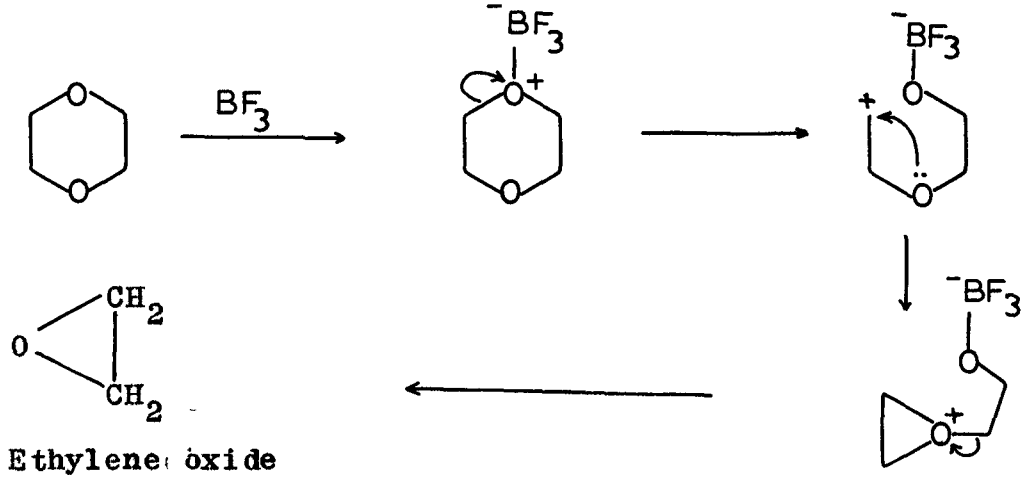
similar to the spectra of the ester (XXIV) but the signal at 6.37 τ (ester protons) was lacking. Further its i.r. spectrum was also identical with that of ester (XXIV) but showed carboxyl absorption (3140, 1710 cm^{-1}). The structure of the acid (XXV) was further supported by mass spectrometry.

Its mass spectrum gave molecular ion peak at m/e 244 ($C_{13}H_{24}O_4$). The other significant peaks obtained were at m/e 243, 227, 73, 60 and 45. The acid (XXV) is split on both sides α to C11 carbon resulting the ions m/e 73 and 243. The preferential formation of the base peak ion m/e 73 over m/e 243 may be attributed to the loss of a bulky group comprising the fatty acid chain. The peaks at m/e 227 and 45 appeared due to fragments arising from α-cleavage with respect to carboxyl group, whereas the peak at m/e 60 is the result of β-cleavage as shown in Scheme - 5.

Scheme - 5.

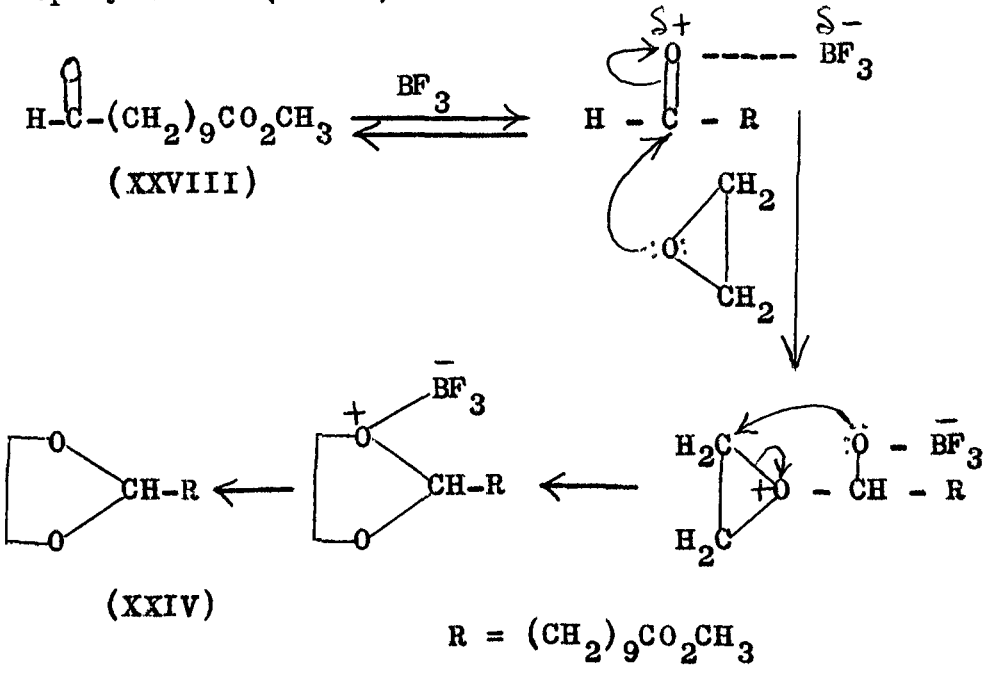


The formation of the ester (XXIV) containing the dioxolane system by the reaction of BF_3-etherate in dioxan with epoxy ester (XXIII) can be attributed to the formation of ethylene oxide during the course of reaction as shown below:



Ethylene oxide

The plausible mechanism leading to the formation of (XXIV) probably involves the reaction of ethylene oxide with the expected rearranged carbonyl derivative (XXVIII) of the parent epoxy ester (XXIII) as outlined below:



(XXVIII)

(XXIV)

$R = (CH_2)_9CO_2CH_3$

Characterization of the Solid (XXVI).

Its identity as methyl 10,11-dihydroxyundecanoate (XXVI) was established by its spectral data and by comparison with an authentic sample. The i.r. of (XXVI) showed the characteristic hydroxyl and ester carbonyl absorptions (3440 and 1740 cm^{-1} respectively). The n.m.r. spectrum showed a three proton unresolved multiplet at 6.6 τ assigned to C10 and C11 protons, as well as signals for two protons at 6.83 τ attributable to hydroxylic protons of the ester (XXVI). The latter signal was extinguished by the addition of deuterium oxide. The methoxy protons as usual appeared as a sharp singlet at 6.35 τ . For the sake of comparison the ester (XXVI) was prepared from 10-undecenoic acid by performic acid hydroxylation using the method of Swern[72]. Performic acid hydroxylation of 10-undecenoic acid yielded 10, 11-dihydroxyundecanoic acid (XXVII) m.p. 85-86^{o} which on methylation by diazomethane yielded the corresponding ester, m.p. 45-46^{o}. This ester showed no depression in mixed melting point with the borontrifluoride catalysed rearranged product (XXVI). Furthermore the i.r. as well as n.m.r. of the two samples of the ester (XXVI) were almost identical in all respects.

The structure of compound (XXVI) was further supported by its mass spectral data. The spectrum showed a weak molecular ion peak at m/e 232 ($C_{12}H_{24}O_4$) and have several other prominent peaks at (m/e 201, 183, 170, 169, 74, 61, 59). The fragment ion

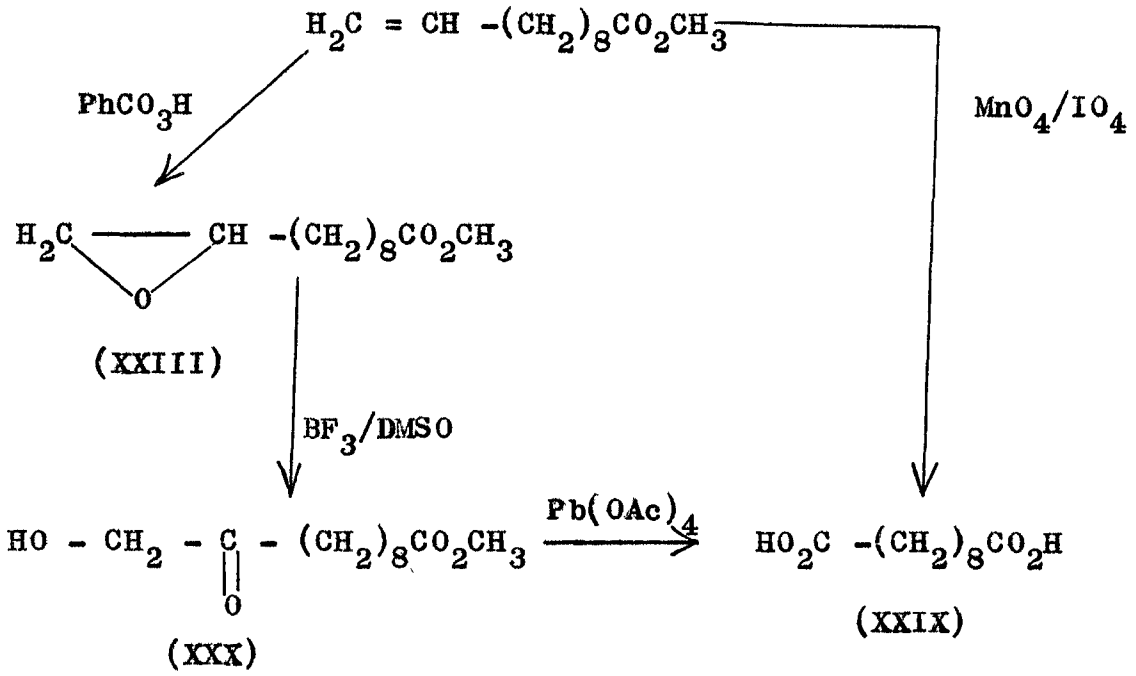
at m/e 201 can be interpreted in terms of the ability of ester to lose 31 (OCH_3) or to the loss of $-CH_2OH$ from the terminal end. This ion m/e 201 on subsequent fragmentation gives ions m/e 169 (base peak) and 183 by the loss of elements of methanol and water molecules respectively. A prominent rearranged ion due to 2,3-cleavage is observed at m/e 74 which is also observed in the spectra of normal long chain methyl esters[73]. Peaks at m/e 59, 61 and 87 are also observed corresponding to ions $\left[O\equiv C-OCH_3\right]^+$, $\left[HO-CH_2-\underset{H}{C}=OH\right]^+$ and $\left[CH_2-CH_2-\overset{O}{\overset{\|}{C}}-OCH_3\right]^+$ respectively.

The work reported earlier show that borontrifluoride catalysed DMSO oxidation of epoxy fatty esters results in the formation of a mixture of two isomeric α-ketol esters which are formed by the opening of the epoxy ring. In the case of the present terminal epoxy ester methyl 10,11-epoxyundecanoate (XXIII) theoretically two products, methyl 10-keto-11-hydroxy and 11-keto-10-hydroxyundecanoates are possible. It was observed that only one α-ketol is exclusively formed in the present reaction. Methyl 10, 11-epoxyundecanoate (XXIII) on treatment with a catalytic amount of BF_3-etherate in DMSO yielded after usual work up[64] a semi-solid product (yield 80-85%). Subsequent purification by column chromatography finally yielded a crystalline t.l.c. homogenous, solid, m.p. 38-39°. It readily formed a DNP derivative melting at 110°. Elemental analysis corresponded to the formula $C_{12}H_{22}O_4$. It was identified as methyl 10-keto-11-hydroxyundecanoate (XXX) by a

study of its spectral data and degradation fragments.

Its i.r. showed peaks at 3440 (OH), 1720 (free C=O), and 1740 cm^{-1} (ester carbonyl). In the n.m.r. it exhibited a one proton singlet centred at 6.7 τ which is assigned to the terminal hydroxyl proton. Addition of D_2O extinguished this signal. A sharp two proton singlet at 5.9 τ appeared which is ascribable to C11 protons of the α-ketol function. The peaks for four protons of α-methylenes adjacent to ketol and ester carbonyls appeared merged as an unsymmetrical quartet observable at 7.71 τ. As the n.m.r. spectrum lacks the formyl proton peak, the presence of the terminal aldehydic function is ruled out. This localizes the ketonic carbonyl at the penultimate carbon (C10). The presence of terminal α-ketol function was established by cleavage with lead tetraacetate. Oxidative degradation of ketol ester (XXX) with lead tetraacetate in acetic acid yielded a fragment which was finally characterized as sebacic acid (XXIX) by its m.p. 134° and co-chromatography with an authentic sample obtained by the permanganate-periodate[74] cleavage of the parent acid (10-undecenoic acid). The formation of sebacic acid as the only fragment of lead tetraacetate cleavage confirms that the carbonyl group is located at C10 of the fatty ester chain. The above reactions are represented in Scheme - 6.

Scheme - 6.



The structure assigned to the ∝-ketol ester, methyl 10-keto-11-hydroxyundecanoate (XXX) was further supported by the study of its mass spectrum. Its mass spectrum gave no molecular ion peak. But a high mass peak at m/e 199 is observed which is ascribable to the loss of methoxy or terminal $-CH_2OH$ group (M-31) from the molecular ion. The other significant peaks obtained were at m/e 171, 139, 121, 74, 69, 59 and 55. The peak at m/e 171 probably arises from the fragment ion m/e 199 by the loss of carbonmonoxide (m/e 199-28) as well as from the molecular ion by the loss of ester moiety (m/e 230-59). The molecule also suffers β-cleavage with respect to the carbonyl groups at C10 acid ester

function giving rise to a peak at m/e 74 of the fragment ions $\left[CH_2=\overset{OH}{\overset{|}{C}}-OCH_3\right]^{+\cdot}$ or $\left[OH-CH_2-\overset{OH}{\overset{|}{C}}=CH_2\right]^{+\cdot}$. A prominent peak at m/e 59 is also observed which may be the result of α-cleavage with respect to C10-carbonyl giving rise to the fragment ion $\left[HO-CH_2-C\equiv O\right]^{+}$.

(C) Studies on the Reaction of Hydrogen bromide in Acetic anhydride with 10,11-dihydroxyundecanoic acid.

Although the bromoacetoxy acids are not classified in the group of oxygenated acids in the usual sence, the importance of these has been realized in the synthesis of oxygenated fatty acids[75]. The bromoacetoxy acids can be prepared by the action of N-bromosuccinimide and acetic acid on olefinic acids or by reaction of hydrogen bromide on epoxy acids followed by acetylation. Another method for the preparation of bromoacetoxy acid first reported by Myers[76] is the action of hydrogen bromide and acetic anhydride on dihydroxy acids derived from unsaturated acids. The literature contains conflicting reports about the interaction of vicinal glycols and hydrogen bromide. Albitzky[77] reported the formation of acetoxybromide, whereas, Baudart and Hunsdiecker[78] claimed the isolation of dibromides. Juleitti and coworkers[79] observed that vicinal dihydroxy acids are readily converted by hydrogen bromide in acetic acid into acetoxybromides which on treatment with alcoholic alkali gave the desired epoxy acid. Myers[76] used hydrogen bromide in acetic anhydride for the preparation of acetoxy derivatives of 2-octadecenoic acid.

Recently Osman and Ahmad[80] have studied the reaction of hydrogen bromide with dihydroxy derivatives of cis- and trans-13-decosenoic (Erucic and brassidic) acids. From the spectral and chemical evidences they have shown that beside the usual

isomeric mixture of bromoacetoxy acids, 13(14), 14(13)-behenic acids a minor product 13(14)-ketobehenic acid is also formed during the course of hydrogen bromide reaction. In order to establish the formation of such unusual reaction product not hitherto reported, Osman and Siddiqui[81] extended the investigation of hydrogen bromide reaction to another pair of isomeric fatty acids, petroselenic and petroselaidic (cis and trans-octadecenoic) acids. In this work also they established the formation of the carbonyl compound, 6(7)-ketostearic acid as a minor product of the reaction of hydrogen bromide on dihydroxy acids.

In the light of facts described above regarding the products of hydrogen bromide reaction on vicinal dihydroxy acids derived from mono unsaturated fatty acids it was considered desirable to study the action of hydrogen bromide in acetic acid on the glycol derivative of a terminal olefinic fatty acid. The results of this study are described in this chapter.

The glycol acid 10,11-dihydroxyundecanoic acid (XXVII) m.p. 85-86°, used in the present reaction, was prepared by performic acid hydroxylation as described earlier[72]. The dihydroxy acid (XXVII) when treated with 48% HBr in acetic anhydride under conditions similar to those of Myers[76] method yielded a brown syrupy liquid. On fractionation over silica gel column this liquid separated into two products (T.L.C. homogenous). These products (XXXI), (XXXII) were identified as 10-acetoxy-11-bromo

(minor) and 10,11-diacetoxyundecanoic (major) acids respectively. A variation in the time of heating (1-5 hrs) did not lead to any other products except the two mentioned above.

The identity of these products was based on chemical and spectral data. The product (XXXI) gave a positive test of halogen and could not be acetylated. Elemental analysis corresponded to the formula $C_{13}H_{23}O_4Br$. Its i.r. gave absorptions at 1235 (acetoxy) and 1740 (acetate carbonyl) and 1710 cm^{-1} (COOH). All these data are consistent with the bromo-acetoxy structure. Reduction of the product (XXXI) with zinc dust in acetone regenerated the original acid (XV) whose n.m.r. showed the typical signals of terminal unsaturated acid, 10-undecenoic acid reported in the literature[82]. The signals obtained were: one proton multiplet 4.32 τ (CH=), two proton doublet, 5.15 τ (=CH_2-) and two proton multiplet at 8.15 τ (CH_2 α to double bond). Its i.r. exhibited the characteristic band of terminal[83] unsaturation at 913 cm^{-1}. When treated with aqueous sodium hydroxide the product (XXXI) instead of yielding the expected bromohydroxy derivative regenerated the glycol acid (XXVII) which was found to be identical with 10,11-dihydroxyundecanoic acid. Confirmation of this was obtained by a comparison of its i.r. and n.m.r. spectra with the spectra of an authentic sample. The mass spectrum of the ester of this dihydroxy acid was also similar to that of methyl 10,11-dihydroxyundecanoate (XXVI) as described earlier. The bromo-

acetoxy derivative (XXXI) after methylation and subsequent catalytic (platinum oxide) hydrogenation yielded a product melting at 49°. It was identified as 10-hydroxyundecanoic acid by comparison of its i.r. and n.m.r. spectra with the spectra of the same acid described during the work on oxymercuration-demercuration. The formation of only 10-hydroxyundecanoic acid localizes the bromine substituent of the bromo-acetoxy product (XXXI) at the terminal carbon (C11). Thus the physico-chemical data established that out of the two isomeric bromo-acetoxy (10-bromo-11-acetoxy and 10-acetoxy-11-bromo) acids, the product (XXXI) is actually the isomer (10-acetoxy-11-bromoundecanoic acid). On the other hand the product (XXXI) on treatment with methanolic potassium hydroxide yielded a crystalline solid (XXXIII), m.p. 58-59°. Its elemental analysis corresponded with the formula $C_{12}H_{24}O_4$. It was characterized as 10-hydroxy-11-methoxyundecanoic acid from its i.r. and n.m.r. spectra. The characteristic bands at 1105 ($-OCH_3$) and 3490 cm^{-1} (OH) were observed in the i.r. spectrum. Its n.m.r. exhibited a three proton sharp singlet at 6.66 τ corresponding to methoxy protons, whereas a downfield two protons triplet (partially merged with $-OCH_3$ signal) centred at 6.79 τ is indicative of the protons of terminal methylene attached to methoxy function. Further the C10-methine proton signal appeare as an unresolved multiplet at 6.29 τ. A two proton broad singlet attributable to free hydroxyl and carboxyl protons appeared at

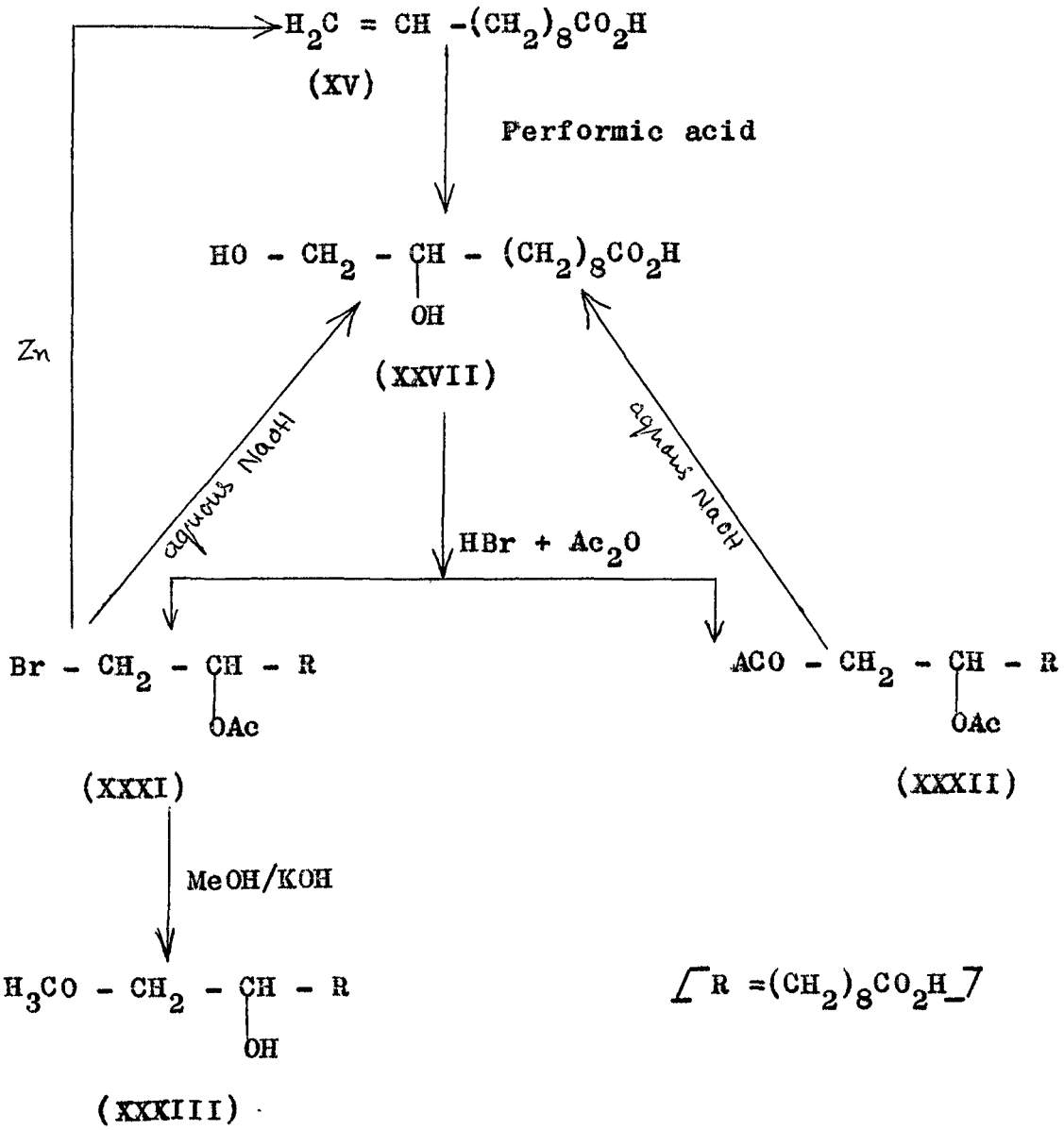
3.15 τ which disappeared after the addition of D_2O. Thus the n.m.r. spectrum was in favour of structure (XXXIII).

The mass spectrum of (XXXIII) showed no molecular ion peak but a small peak at m/e 187 is observed which is ascribable to the loss of the fragment (CH_2OCH_3) from the molecular ion. The other prominent peaks observed are at m/e 170, 169, 81, 75, 67, 60, 59, 55, 45. The ion m/e 187 further loses a molecule of water to give rise m/e 169. The peaks at m/e 75 and m/e 45 (base peak) may be assigned to the fragment ions $[CH_3O-CH_2-CH=OH]^+$ and $[CH_2=OCH_3]^+$ respectively. A prominent rearranged ion due to 2,3-cleavage is observed at m/e 60 which is a common feature in the mass spectra of fatty acids.

The major product (XXXII) was identified as 10,11-diacetoxyundecanoic acid by co-chromatography (TLC) with an authentic sample prepared by the acetylation of 10,11-dihydroxy-undecanoic acid. Its elemental analysis corresponded to the formula $C_{15}H_{26}O_6$. It showed negative test for halogen and did not react with Jones reagent as well as with acetic anhydride-pyridine. But treatment with aqueous sodium hydroxide yielded the desired glycol acid (XXVII), whose i.r. and n.m.r. spectra were found to be almost identical with those of 10,11-dihydroxy-undecanoic acid.

The foregoing reactions are represented as shown in the Scheme - 7.

Scheme - 7.


H2C = CH -(CH2)8CO2H
(XV)
Performic acid
HO - CH2 - CH - (CH2)8CO2H
OH
(XXVII)
Zn
aqueous NaOH
aqueous NaOH
HBr + Ac2O
Br - CH2 - CH - R
OAc
(XXXI)
ACO - CH2 - CH - R
OAc
(XXXII)
MeOH/KOH
H3CO - CH2 - CH - R
OH
(XXXIII)
[R =(CH2)8CO2H]

E X P E R I M E N T A L

EXPERIMENTAL

All melting points are uncorrected. I.r. spectra were obtained with Perkin-Elmer 237 Spectrophotometer. N.m.r. spectra were run in $CDCl_3$ on Varian A60/HA100 with TMS as the internal standard. Mass spectra were measured in an AEI MS-9 mass spectrometer using a direct insertion sample inlet system. Thin layer chromatographic plates were coated with silica gel G and chromic acid (50%) was used as spraying agent. Pet. ether refers to a fraction b.p. 60-80°. N.m.r. and Mass data are given in discussion part of the present work.

(A) Studies on Solvomercuration and Demercuration of 10-undecenoic Acid.

Preparation of 10-hydroxyundecanoic acid (XVI).

In a conical flask fitted with a magnetic stirrer was placed mercuric acetate (3.19 g, 10.0 m. mol). Water (10 ml) was added to dissolve the mercuric acetate followed by tetrahydrofuran (10 ml). Then undecenoic acid (1.84 g, 10 m. mol) was added. The yellow color disappeared in 15-20 seconds. The reaction mixture was stirred for 20 minutes at room temperature ($\sim 25^{\circ}$) to complete the oxymercuration stage. Then caustic soda (10.0 ml; 2.0M) was added, followed by sodium borohydride solution (10.0 ml; 0.5M) in NaOH (3.0M). After the reduction at 30° the mercury was allowed to settle. Sodium chloride was added to

saturate the water layer. The reaction mixture was acidified with dil. HCl to regenerate the acids, as well as to destroy the excess of sodium borohydride. The product on extraction twice with ether, dried (anhydrous Na_2SO_4) and evaporated to dryness, yielded a viscous oil (1.82 g). This was crystallized twice from pet. ether-ether (1:1) to furnish white crystals of 10-hydroxyundecanoic acid (XVI) (1.54 g) m.p. and m.m.p. 49° (lit.[84] m.p. 49.5°), (Found: C, 65.32; H, 10.95. Calcd. for $C_{11}H_{22}O_3$: C, 65.31; H, 10.96%); i.r. (KBr) 3440 (-OH) cm^{-1}.

Oxidation of 10-hydroxyundecanoic acid (XVI) with Jones reagent.

The hydroxy acid (1 g) was dissolved in acetone (50 ml) and the solution cooled to 10-15° in a cold bath. Jones reagent (4 ml) was added dropwise over a period of 20 minutes with continuous shaking of the reaction mixture. After the addition was complete the reaction mixture was allowed to stand at 15-20° for 1 hr. The mixture was poured in cold water, extracted with ether and dried (anhydrous Na_2SO_4). Evaporation of the solvent gave the 10-ketoundecanoic acid (XVII) as a solid (0.85 g) which was crystallized in pet. ether-benzene (1:1) to yield shining crystals m.p. 56-57° (lit.[85] m.p. 59°); (Found: C, 65.89; H, 10.07. Calcd. for $C_{11}H_{20}O_3$: C, 65.97; H, 10.07%); i.r. (KBr) 1720 (C=O) cm^{-1}.

Derivatization of 10-ketoundecanoic acid (XVII).

(a) 2,4-Dinitrophenyl osazone. The keto acid (50 mg) obtained from the oxidation of 10-hydroxyundecanoic acid (XVI) was added to a clear solution of 2,4-dinitrophenyl hydrazine (0.1 g in 4 ml methanol and one drop of sulphuric acid). The mixture was heated to boiling and allowed to cool. The solid product when worked up as usual gave 2,4-dinitrophenyl hydrazone crystallized from ethanol in the form of shining yellow crystals, yield (0.35 mg), m.p. 115-116°, (Found: C, 53.65; H, 6.34; N, 14.65. $C_{17}H_{24}N_4O_6$ requires C, 53.67; H, 6.36; N, 14.73%).

(b) Semicarbazone. The keto acid (XVII) (0.2 g) was added to an aqueous solution of semicarbazide hydrochloride (0.4 g) and sodium acetate (0.6 g) and shaken vigorously. The resulting solid product was filtered, washed with cold water and on crystallization twice with ethanol, yielded a semicarbazone, m.p. 136° (lit.[85] m.p. 136°), (Found: C, 56.1; H, 8.8; N, 16.4. Calcd. for $C_{12}H_{13}O_3N_3$: C, 56.0; H, 8.9; N, 16.3%).

Partial Synthesis of 10-hydroxyundecanoic acid (XVI).

(a) Preparation of 10,11-dibromoundecanoic acid: To a cooled solution of undecenoic acid (12 g) in chloroform (30 ml), bromine

(6 g) was added dropwise. When the addition was over the mixture was stirred for 3 hrs and washed with sodium thiosulphate solution (5%) and water. The chloroform solution was dried and the product taken up with ether. Evaporation of solvent yielded dibromoundecanoic acid (17 g) as a thick viscous liquid.

(b) Dehydrobromination of 10,11-dibromoundecanoic acid. 10,11-dibromoundecanoic acid (9 g), potassium hydroxide (18 g), water (5 ml) and ethanol (75 ml) were taken in a 250 ml round bottomed flask and refluxed on a water bath for 8 hrs. Most of the alcohol was removed under reduced pressure, and the product dissolved in water and acidified with cold dil. sulphuric acid. This was extracted with ether, washed, and dried. Evaporation of the solvent yielded a semi-solid (a single spot on t.l.c.) which on crystallization from pet. ether afforded pure 10-undecynoic acid (XVIII), m.p. 41-42° (lit.[86] m.p. 43°), (Found: C, 72.47; H, 9.89. Calcd. for $C_{11}H_{18}O_2$: C, 72.49; H, 9.96%); i.r. ($CHCl_3$) 2122 (-C≡C-) cm^{-1}.

(c) Hydration of 10-undecynoic acid[57]. 10-undecynoic acid (6 g) was taken in a conical flask cooled in ice-salt mixture and ice-cold sulphuric acid (45 ml) added dropwise with frequent shaking. When the addition was over, the flask was kept at room temperature overnight. The contents were poured in ice-cold

water, washed free of acid and dried to give a white solid (5.3 g) which was crystallized in pet. ether and benzene (1:1) to give shining crystals of 10-keto-undecanoic acid (XVII), m.p. and m.m.p. 56-57° (lit.[85] 59°).

(d) $NaBH_4$ reduction of 10-ketoundecanoic acid (XVII). To a solution of 10-ketoundecanoic acid (XVII) (0.2 g) in methanol (30 ml), a solution of sodium borohydride (0.6 g) in methanol (20 ml) was added. After the initial vigorous reaction had subsided, the mixture was refluxed for 3 hours, acidified with dil. hydrochloric acid, diluted with cold water and extracted repeatedly with ether. Combined extracts were dried and then evaporated to yield crude 10-hydroxyundecanoic acid (0.17 g) which was crystallized in pet. ether-ether (1:1) to give pure 10-hydroxyundecanoic acid (XVI) m.p. and m.m.p. 49°.

Preparation of 10-aminoundecanoic acid.

Mercuric nitrate (5.2 g) and methyl cyanide (8 ml) were taken in a conical flask. To the solution undecenoic acid (2.94 g) was added and the contents were stirred at room temperature for 1 hr. The stirring was done at such a rate as to maintain the temperature below 30°C. The mixture was cooled and the reduction achieved by adding NaOH (16 ml; 2.0 M), followed

by sodium borohydride (16 ml; 0.5M) NaOH (3.0 M). After 1 hr the solution was acidified by dil. sulphuric acid and extracted twice with ether and dried. The ether was then evaporated to yield an oil (2.9 g) which was chromatographed over a column of silica gel (60 g). Elution with pet. ether with increasing amount of ether furnished, a t.l.c. homogeneous solid (2.1 g) which was crystallized in pet. ether-ether (2:3) to furnish pure 10-aminoundecanoic acid, m.p. 38-40°, (Found: C, 65.54; H, 11.52; N, 6.88. $C_{11}H_{23}O_3N$ requires C, 65.63; H, 11.52; N, 6.96%); i.r. ($CHCl_3$) 3415 ($-NH_2$) cm^{-1}.

Oxidation of 10-aminoundecanoic acid.

50 mg. of amino acid was oxidized at 0-5°C with Jones reagent essentially as described earlier. After the usual work up the amino compound was recoverred as such.

Acid hydrolysis of 10-aminoundecanoic acid.

10-Aminoundecanoic acid (0.3 g) was dissolved in dil. hydrochloric acid (25 ml) and the solution was refluxed for one hour, cooled and extracted with ether, washed with water and dried. The ether was evaporated to yield a solid product (0.26 g) which was crystallized in pet. ether-ether (1:1) to yield a white

crystalline 10-hydroxyundecanoic acid m.p. and m.m.p. 49°.

Oxidation of hydrolysed product of 10-aminoundecanoic acid.

Hydrolysed product (100 mg) was oxidized with Jones reagent to yield a solid product (65 mg) which was crystallized in pet. ether-benzene (1:1) to give 10-ketoundecanoic acid (XVII) m.p. and m.m.p. 56-57°.

Preparation of 10-methoxy and 10-ethoxyundecanoic acid.

Anhydrous methanol (50 ml) and mercuric acetate (15.95 g) were taken in a conical flask. To the vigorously stirred solution undecenoic acid (9.2 g) was added. The reaction mixture was stirred for 15 minutes. Then the reduction of the mercurial intermediate was achieved by adding NaOH (50 ml; 3.0 M) and sodium borohydride (50 ml; 0.5 M) in NaOH (3.0 M) both in water. The mixture was stirred for another two hours, until the mercury had coagulated and settled down. After acidification with dil. hydrochloric acid, it was extracted with ether, dried. Evaporation of ether yielded an oil (9.3 g). The oily product when chromatographed over silica gel (200 g) and eluted with pet. ether and ether (100:14; V/V) which furnished pure 10-methoxyundecanoic acid (8.28 g) as a colorless oil. TLC analysis showed

a single component. (Found: C, 66.64; H, 11.17. Calcd. for $C_{12}H_{24}O_3$: C, 66.63; H, 11.18%); i.r. (neat) 1110 (C-O-) cm^{-1}.

A similar reaction of undecenoic acid (5.0 g) with ethanol (27 ml) and mercuric acetate (8.7 g) and subsequent reduction gave 10-ethoxyundecanoic acid (yield $\sim$ 90%), (Found: C, 67.69; H, 11.38. $C_{13}H_{26}O_3$ requires C, 67.78; H, 11.38%); i.r. (neat) 1110 (C-O-) cm^{-1}. The use of isopropanol as solvent did not bring about etherification as shown by t.l.c.

Preparation of hydroxy ethers (XIX) and (XX).

Ethylene glycol (12.5 ml) and mercuric acetate (3.9 g) were taken in a flask. The solution was slightly heated to dissolve the mercuric acetate and then vigorously stirred. Undecenoic acid (2.3 g) was introduced into vigorously stirred solution. The reduction of the mercurial intermediate was achieved as usual by adding NaOH (12.5 ml; 3.0 M) and $NaBH_4$ (12.5 ml; 0.5 M) in NaOH (3.0M). The usual work up of the reaction mixture and chromatographic separation yielded 10-(2'-hydroxyethoxy)undecanoic acid (XIX) as a t.l.c. homogeneous oil (2.59). (Found: C, 63.39; H, 10.66. $C_{13}H_{26}O_4$ requires C, 63.38; H, 10.64%); i.r. (neat) 3410 (OH), 1110, 1056 (C-O-) cm^{-1}.

Propylene glycol (25 ml), mercuric acetate (7.8 g), and

undecenoic acid (4.6 g) were reacted as described above. A similar reduction of the mercurial intermediate finally gave 10-(2'-methyl-2-hydroxyethoxy)undecanoic acid (XX) as an oily product (4.8 g). (Found: C, 64.57; H, 10.84. $C_{14}H_{28}O_4$ requires C, 64.58; H, 10.84%); i.r. (neat) 3410 (OH), 1110, 1056 (C-O-) cm^{-1}.

Attempted Jones oxidation of hydroxy ethers (XIX) and (XX).

The hydroxy ether (XIX) (1 g) was dissolved in acetone (50 ml) and the solution cooled to 10-15° in a cold water bath. Jones reagent (4 ml) was added dropwise with continuous shaking. After the addition was complete, the reaction mixture was allowed to stand at 15-20° for half an hour. The mixture was poured in cold water, extracted with ether and dried. Evaporation of the solvent gave a viscous colorless oil (0.71 g). The oil was chromatographed over silica gel (20 g). Elution with pet. ether-Ether (100:18; V/V) gave a keto acid as a solid which crystallized in benzene-pet. ether (1:1) to yield (0.49 g) shining crystals of 10-ketoundecanoic acid (XVII) m.p. and m.m.p. 56-57°. Co-chromatography (t.l.c.) with authentic 10-ketoundecanoic acid showed a single spot. I.r. spectra superimposable with the spectra of 10-ketoundecanoic acid.

The hydroxy ether (XX) on Jones oxidation under similar

conditions also yielded a product m.p. 56-57°. It showed no depression in m.p. with authentic 10-ketoundecanoic acid. Mobility of the two compounds on t.l.c. was identical.

The keto compound, obtained by Jones oxidation of hydroxy ethers (XIX) and (XX), gave a 2,4-dinitrophenyl hydrazone m.p. and m.m.p. 115-116° and a semicarbazone m.p. and m.m.p. 136°.

Potassium permanganate oxidation of hydroxy ether (XIX).

A portion of hydroxy ether (XIX) (0.75 g) was dissolved in water (40 ml) and potassium permanganate (1.2 g) and sodium hydroxide (10%; 1.6 ml) was added with stirring at room temperature. After 30 minutes at room temperature and 2 hrs at 35°, the excess of potassium permanganate was destroyed by addition of sodium bisulphite. After decomposition by dil. hydrochloric acid the product was extracted repeatedly with ether. Ether evaporation yielded an oil (0.42 g). TLC showed a little amount of unreacted material. The reaction product was separated by preparative t.l.c. using Benzene-methol (90:10; V/V) to yield t.l.c. pure carboxy ether (XXI) as a colorless oil. (Found: C, 59.89; H, 9.28. $C_{13}H_{24}O_5$ requires C, 59.98; H, 9.29%); i.r. (neat) 1705 (COOH), 1110, 1050 (C-O-) cm^{-1}.

Chromium trioxide-pyridine[87] oxidation of hydroxy ether (XIX).

A slurry of the complex in anhydrous pyridine was prepared by adding chromium trioxide (0.14 g) to vigorously stirred chilled (ice bath) anhydrous pyridine (1.4 ml) over a period of 10-15 minutes. 10-(2'-Hydroxyethoxy)undecanoic acid (XIX) (0.14 g) in pyridine (0.7 ml) was added to the slurry in portion. The mixture was allowed to react 24 hrs at room temperature with continuous stirring. The mixture was then diluted with water and extracted three times with ether. The combined ether extracts were successively washed with 5% HCl and water. Evaporation of dried ethereal extract yielded a dark reddish product (0.1 g). Fractionation by preparative t.l.c. gave the carboxy ether (XXI) as a noncrystallizable colorless oil.

Chromium trioxide-pyridine oxidation of hydroxy ether (XX).

The chromium trioxide oxidation of 10-(2'-methyl-2-hydroxyethoxy)undecanoic acid (XX) was essentially carried out as described earlier using the same quantity of hydroxy ether and reagents to yield a dark viscous reddish oil. Purification by column chromatography over silica gel gave a viscous oil (XXII) which resisted all attempts to crystallization. (Found: C, 65.10; H, 10.14. $C_{14}H_{26}O_4$ requires C, 65.08; H, 10.14%); i.r. (neat) 1715 (C=O), 1112, 1050 (C-O-) cm^{-1}.

(B) Boron trifluoride catalysed reactions of methyl 10, 11-epoxyundecanoate.

Preparation of Methyl 10-undecenoate.

10-undecenoic acid (10 g) was esterified with methanolic sulphuric acid solution (2.5%; 60 ml) by refluxing for 1 hr. After the usual work up evaporation of ether yielded methyl 10-undecenoate as a colorless oil (9.1 g).

Preparation of Methyl 10, 11-epoxyundecanoate (XXIII).

Methyl undecenoate (9.1 g; 0.046 mole) was epoxidized with 105.9 ml (0.46 mole) of perbenzoic acid solution at 5^{o}. The solvent was removed under reduced pressure and the residue taken up with ether. The ether extract was washed with sodium bicarbonate solution (4%) and then with water. Evaporation of dried ethereal solution yielded a colorless oil (8.1 g). TLC as well as picric acid TLC^{70} of the oil showed almost complete conversion of unsaturated acid ester to methyl 10, 11-epoxy-undecanoate (XXIII) (Found: C, 67.26; H, 10.34. Calcd. for $C_{12}H_{22}O_3$: C, 67.25; H, 10.35%); i.r. (neat) 1740 (ester carbonyl) 830 (epoxy) cm^{-1}. (Epoxy oxygen found 7.38, Calcd. 7.48%).

Reaction of Methyl 10, 11-epoxyundecanoate (XXIII) with Boron Trifluoride etherate[58].

Methyl epoxyundecanoate (XXIII) (1 gm; 4 m. mole) was refluxed for 3 hrs in anhydrous dioxan (74 ml) with boron trifluoride etherate (0.7 ml). The product extracted with ether after dilution with water, and dried yielded an oily substance (0.8 g). Direct t.l.c. showed two distinct spots.

Separation procedure.

The oily product (0.8 g) was chromatographed on silica gel G (20 g) and the elution was carried out with pet. ether containing increasing amounts of ether. Eluted material was monitored by t.l.c. using pet. ether-ether as solvent. Elution with pet. ether-ether (88:12; V/V) yielded methyl 11, 11-ethylenedioxyundecanoate (XXIV) as a viscous liquid (0.61 g). It resisted all attempts to crystallization. (Found C, 65.07; H, 10.14. $C_{14}H_{26}O_4$ requires C, 65.08; H, 10.14%); i.r. (neat) 1740 (ester carbonyl), 1110, 1040, 1225, 1255 (C-O-) cm^{-1}. Fractions collected by elution with pet. ether-ether mixture (65:35; V/V) gave a solid which was crystallized in benzene to give shining crystals of methyl 10, 11-dihydroxyundecanoate (XXVI), m.p. and m.m.p. 45-46° (lit.[88] m.p. 46.5-47°), (Found: C, 62.03; H, 10.42. Calcd. for $C_{12}H_{24}O_4$: C, 62.04; H, 10.41%); i.r. ($CHCl_3$) 3440 (OH), 1740 (ester carbonyl) cm^{-1}.

Hydrolysis of product (XXIV) with methanolic KOH.

The product (XXIV) (0.4 g) was refluxed on water bath for half an hour with 8% methanolic potassium hydroxide (4 ml). The reaction mixture was cooled to 0°C and acidified by addition drop by drop of cold 5% dil. hydrochloric acid. The solid product (0.2 g) was filtered, washed and dried. It was crystallized in pet. ether to yield t.l.c. pure 11, 11-ethylenedioxyundecanoic acid (XXV) m.p. 66-68°. (Found: C, 63.86; H, 9.91. $C_{13}H_{24}O_4$ requires C, 63.90; H, 9.90%); i.r. (KBr) 3140, 1710 (COOH) cm^{-1}.

Preparation of Methyl 10, 11-dihydroxyundecanoate (XXVI).

Undecenoic acid (2 g) was taken up in formic acid (6 ml) and was oxidized with hydrogen peroxide (1.4 g; 30%) at 25°. The temperature was not allowed to exceed beyond 40°C throughout the reaction time (4 hrs). The reaction mixture was poured into cold water and worked up according to the procedure of . . Swern[72]. The crude hydroxylation product (1.5 g) when crystallized in acetone yielded pure 10, 11-dihydroxyundecanoic acid (XXVII), m.p. 86-86° (lit.[72] m.p. 85-86°).

The above dihydroxy acid was dissolved in absolute methanol and treated with diazomethane. The usual work up afforded a solid

which was crystallized in benzene to give shining crystals of methyl 10, 11-dihydroxyundecanoate (XXVI), m.p. 45-46° (lit.[88] m.p. 46.5-47°).

Dimethyl Sulfoxide[65] oxidation of methyl 10, 11-epoxyundecanoate (XXIII).

A solution of methyl 10, 11-epoxyundecanoate (2.8 g) and boron trifluoride etherate (2 drops) in predried dimethyl sulfoxide (12 ml) was heated on a water bath for 22 hrs. Additional portion (1 drop) of the catalyst was added at the end of 15th hr. The reaction mixture was poured into ice-cold water and extracted with chloroform. Evaporation of dried extract yielded a semi-solid (2.34 g) which was purified by column chromatography over silica gel (40 g) using pet. ether-ether (100:20; V/V) to give a solid compound. Crystallization with pet. ether-ether (1:1) yielded t.l.c. pure methyl 10 keto-11-hydroxyundecanoate (XXX), m.p. 38-39°. (Found: C, 62.59; H, 9.63. $C_{12}H_{22}O_4$ requires: C, 62.58; H, 9.63%); i.r. ($CHCl_3$) 3440 (OH), 1720 (free C=O), 1740 (ester carbonyl) cm^{-1}.

2,4-Dinitrophenyl Osazone.

The ketol ester (XXX) (50 mg) was added to a solution of

2,4-dinitrophenyl hydrazine (0.1 g in 4 ml methanol and one drop of sulphuric acid). The usual work up resulted a product which on crystallization with ethanol finally yielded yellow crystals of 2,4-dinitrophenyl hydrazone, m.p. 110°. (Found: C, 56.65; H, 6.35; N, 13.63. $C_{18}H_{26}N_4O_7$ requires C, 56.67; H, 6.39; N, 13.65%).

Characterization of product (XXX) by degradation with lead tetraacetate.

Lead tetraacetate used in the cleavage of ∝ -ketol ester was prepared by allowing dry lead (22 g) to react with acetic anhydride (12.5 ml) and acetic acid (18.2 ml) according to the standard procedure[89]. The degradation of ∝ -ketol ester (XXX) was carried out according to Baer's procedure[90] as follows:

A solution of methyl 11-hydroxy-13-ketoundecanoate (XXX) (1 g), m.p. 38-39°, in 70% acetic acid (30 ml) was treated with powdered lead tetraacetate (3.2 g). The mixture gradually turned black on shaking for about 30 minutes. After the removal of excess of lead and acetic acid, the concentrate on cooling gave a semi-solid substance which was hydrolysed by aqueous NaOH and filtered, washed and dried. This on crystallization from acetone afforded sebacic acid (XXIX), m.p. and m.m.p. 134° (lit.[91] 134°). Co-chromatography with a pure specimen of

sebacic acid showed only one peak. (Found: C, 59.37; H, 8.97. Calcd. for $C_{10}H_{18}O_4$ C, 59.38; H, 8.97%).

Periodate-permanganate[74] oxidation of 10-undecenoic acid (XV).

The undecenoic acid (0.5 g) dissolved in tertiary butanol (100 ml) was added to oxidant solution (120 ml) prepared by dissolving sodium periodate (1.769 g) and potassium permanganate (0.02 g) in water (400 ml). To the mixture was then added 25 ml potassium carbonate (270 mg) and butanol (100 ml). The contents were stirred for 3 hrs at 65°. Butanol was evaporated on a water bath under nitrogen. The contents were heated with few pellets of caustic potash. The saponified product was acidified with sulphuric acid (10%) to yield a solid product which was filtered, washed, dried and crystallized in acetone to give sebacic acid (XXIX) m.p. 134° (m.p. undepressed with admixture with the sample obtained from lead tetraacetate cleavage).

(C) Studies on The Reaction of Hydrogen bromide in Acetic anhydride with 10, 11-dihydroxyundecanoic acid.

Reaction of HBr and Ac_2O on 10, 11-dihydroxyundecanoic acid (XXVII).

The dihydroxy acid (10 g, prepared as described earlier)

dissolved in acetic anhydride (160 ml) was reacted with 48% hydrogen bromide (53 g) added dropwise. The temperature was maintained below 40^{o} by cooling with ice water. The contents were then heated for 2 hrs at ($80-88^{o}$). The product when worked up following the procedure of Myer[76] yielded a syrupy liquid (11.6 g).

Separation procedure of bromoacetoxy (XXXI) and diacetoxy undecanoic (XXXII) acids.

The liquid product (11.6 g) was chromatographed over a column of silica gel. TLC monitored elution with a mixture of pet. ether-ether (100: 15; V/V) gave bromoacetoxy acid (XXXI) in the form of light brown liquid (2.8 g). It gave positive test for bromine and resisted acetylation. (Found: C, 48.1; H, 7.1. $C_{13}H_{23}O_4Br$ requires C, 48.2; H, 7.1%); i.r. (neat) 1740 (*acetate carbonyl*), 1235 (-O-C-).

Elution with pet. ether-ether (100:18; V/V) gave diacetoxyundecanoic acid (XXXII) as a colorless oil (7.4 g). It showed a negative test for halogen. It resisted attempts to acetylation and oxidation by Jones reagent. (Found: C, 59.4; H, 8.6. Calcd. for $C_{15}H_{26}O_6$ C, 59.6; H, 8.6%); i.r. (neat) 1740 (acetate carbonyl), 1710 (COOH), 1235 (-O-C-) cm^{-1}.

Attempted de-acetylation of bromoacetoxy undecanoic acid (XXXI) by methanolic KOH.

The bromoacetoxyundecanoic acid (XXXI) (0.25 g) was refluxed for 2 hrs with 10% methanolic potassium hydroxide (10 mole). The solvent was removed under reduced pressure and the residue cooled, diluted with water and acidified with dil. hydrochloric acid. The resultant solid product was filtered and dried. Crystallization with 2:1 mixture of pet. ether-ether gave 10-hydroxy-11-methoxyundecanoic acid (XXXIII), m.p. 58-59^{o} (yield 0.16 g). This compound did not respond to halogen test and could be acetylated. (Found: C, 62.05; H, 10.42. $C_{12}H_{24}O_4$ requires C, 62.04; H, 10.41%); i.r. (KBr) 3490 (OH), 1105 ($-OCH_3$) cm^{-1}.

Aqueous alkaline hydrolysis of Bromoacetoxyundecanoic acid (XXXI).

Bromoacetoxyundecanoic acid (XXXI) (0.25 g) was hydrolyzed by refluxing for one hour with aqueous sodium hydroxide (2.5 ml; 0.5N). The reaction mixture was cooled and acidified by dil. hydrochloric acid to yield a solid which was filtered, dried and crystallized in acetone to give 10, 11-dihydroxyundecanoic acid (XXVII) m.p. and m.m.p. 86-87^{o}. This could be acetylated but gave a negative test for halogen. Co-chromatography with

the authentic dihydroxyundecanoic acid gave a single spot. *Diazomethane treatment* gave the dihydroxy acid ester (XXVI) m.p. and m.m.p. 45-46°.

Reduction of Bromoacetoxyundecanoic acid (XXXI).

10-Acetoxy-11-bromoundecanoic acid (XXXI) (1 g) dissolved in acetone (5 ml), was reduced with zinc dust (1.1 g) by refluxing for 16 hrs with occasional stirring. The contents were filtered and the filtrate acidified with dil. hydrochloric acid and extracted twice with ether. The combined ether extracts were washed and dried. Evaporation of the solvent gave a colorless oil which on crystallization with n-hexane at -18° yielded shining leaflets of 10-undecenoic acid (XV), m.p. and m.m.p. 24° (lit.[92] 24-24.5°). Hydroxylation with perfomic acid as described earlier[72] afforded 10,11-dihydroxyundecanoic acid m.p. and m.m.p. 86-87°.

Conversion of Bromoacetoxyundecanoic acid (XXXI) to 10-hydroxyundecanoic acid (XVI)[93].

A solution of bromoacetoxy acid (XXXI) (0.6 g) in methanol (80 ml) containing concentrated sulphuric acid (2.1 ml), was refluxed for 10 hours. Most of the methanol was removed under

reduced pressure and the concentrate was then diluted with water, extracted with ether and washed. The residue obtained by evaporation was dissolved in ethanol (20 ml) containing Adams platinum oxide catalyst (0.6 g) and shaken with hydrogen, under slight pressure for 4 days. The catalyst was then removed by filtration and the filtrate was diluted with aqueous sodium hydroxide (10 ml; 10%) and refluxed for 1 hour. This hydrolysate was acidified and extracted with ether. Crystallization of the product with pet. ether-ether (1:1) yielded pure 10-hydroxyundecanoic acid (XVI), m.p. and m.m.p. 49°.

Alkaline hydrolysis of diacetoxyundecanoic acid (XXXII).

Diacetoxyundecanoic acid (XXXII) (1 g) was hydrolysed by refluxing for one hour with aqueous sodium hydroxide (20 ml; 0.5N). After the usual work up the products on crystallization from acetone afforded 10,11-dihydroxyundecanoic acid (XXVII) m.p. and m.m.p. 85-86°. This dihydroxy acid on acetylation regenerated the original 10,11-diacetoxyundecanoic acid (XXXII).

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